**ISSN** 2150-5330 (online)

# World Journal of *Gastrointestinal Pathophysiology*

*World J Gastrointest Pathophysiol* 2017 May 15; 8(2): 11-99

Published by Baishideng Publishing Group Inc

WJGP World Journal of Gastrointestinal Pathophysiology

A peer-reviewed, online, open-access journal of gastrointestinal pathophysiology

# Editorial Board

2016-2019

The *World Journal of Gastrointestinal Pathophysiology* Editorial Board consists of 503 members, representing a team of worldwide experts in gastrointestinal pathophysiology. They are from 45 countries, including Argentina (2), Australia (13), Austria (2), Belgium (7), Brazil (9), Brunei Darussalam (1), Canada (21), China (30), Croatia (1), Czech Repoublic (2), Denmark (3), Egypt (1), Estonia (1), Finland (1), France (8), Germany (24), Greece (7), Hungary (5), India (12), Indonesia (1), Iran (2), Ireland (2), Israel (8), Italy (38), Japan (45), Lebanon (3), Malaysia (1), Mexico (2), Netherlands (7), Norway (1), Poland (4), Portugal (1), Romania (1), Russia (1), Singapore (4), South Korea (12), Spain (24), Sweden (11), Switzerland (3), Thailand (2), Turkey (7), Ukraine (1), United Kingdom (10), United States (161) and Venezuela (1).

Zhan-Ju Liu, *Shanghai*
Shi Liu, *Wuhan*
Zhen-Ning Wang, *Shenyang*
Xiao-Hong Wang, *Beijing*
Wei Wei, *Hefei*
Dong-Ping Xie, *Shanghai*
Wen-Xie Xu, *Shanghai*
Xiao Yang, *Beijing*
Hua Yang, *Chongqing*
Wei-Zhen Zhang, *Beijing*
Hua-Chuan Zheng, *Shenyang*
Min-Sheng Zhu, *Nanjing*
Jinxia Zhu, *Beijing*
Da-Ling Zhu, *Harbin*
Yong-Liang Zhu, *Hangzhou*

**Croatia**

Alen Protic, *Rijeka*

**Czech Republic**

Pavel Hladik, *Semily*
Martin Vokurka, *Prague*

**Denmark**

Lars Arendt-Nielsen, *Aalborg*
Frank V Schiodt, *Copenhagen*
Jonas Worsoe, *Aarhus*

**Egypt**

Mahmoud Aboelneen Khattab, *Minia*

**Estonia**

Enn Seppet, *Tartu*

**Finland**

Pauli Antero Puolakkainen, *Turku*

**France**

Bruno Bonaz, *Grenoble*
Pierre M Dechelotte, *Rouen*
Jean-Paul Lallès, *Saint-Gilles*
Charles-Henri Malbert, *Saint-Gilles*
Thierry Piche, *Nice*
Pascale Plaisancié, *Lyon*
Michelina Plateroti, *Lyon*
Véronique Vitton, *Marseille*

**Germany**

Hans G Beger, *Ulm*
Carsten Bergmann, *Ingelheim*
Elke Cario, *Essen*
Arno J Dormann, *Koln*
Nikolaus Gassler, *Aachen*
Andreas Geier, *Wuerzburg*
Werner Hartwig, *Heidelberg*
Marion Hewicker-Trautwein, *Hannover*
Jens Hoeppner, *Freiburg*
Tobias Keck, *Freiburg*
Jorg Kleeff, *Munich*
Peter Malfertheiner, *Magdeburg*
Oliver Mann, *Hamburg*
Christoph W Michalski, *Munich*
Andreas K Nussler, *Munich*
Christian Pehl, *Vilsbiburg*
Peter Schemmer, *Heidelberg*
Manuel A Silva, *Penzberg*
Marc Stemmler, *Freiburg*
Frank Tacke, *Aachen*
Sya N Ukena, *Braunschweig*
Brigitte Vollmar, *Rostock*
Thomas M Wex, *Magdeburg*
Margot Zoller, *Heidelberg*

**Greece**

Stelios F Assimakopoulos, *Patras*
George N Dalekos, *Larissa*
Alkiviadis Efthymiou, *thessaloniki*
Maria Gazouli, *Athens*
Ioannis E Koutroubakis, *Heraklion*
Gerassimos J Mantzaris, *Athens*
George V Papatheodoridis, *Athens*

**Hungary**

Mária Bagyánszki, *Szeged*
Mihály Boros, *Szeged*
Laszlo Czako, *Szeged*
Pal Miheller, *Budapest*
Zoltan Rakonczay, *Szeged*

**India**

Anil K Agarwal, *Delhi*
Uday Bandyopadhyay, *Kolkata*
Sriparna Basu, *Varanasi*
Chandra K Chakraborti, *Rourkela*
Nilesh M Dagia, *Mumbai*
Rajeev Garg, *Punjab*
Akhtar Mahmood, *Chandigarh*
Veerareddy P Reddy, *Andhra Pradesh*
Chandra P Sharma, *Thiruvananthapuram*
Shailesh V Shrikhande, *Mumbai*
Virendra Singh, *Chandigarh*
Nicholas J Skill, *Indianapolis*

**Indonesia**

Laurentius A Lesmana, *Jakarta*

**Iran**

Gholamreza Roshandel, *Gorgan*
Shahram Shahabi, *Urmia*

**Ireland**

Billy Bourke, *Dublin*
Stephen J Keely, *Dublin*

**Israel**

Yosefa Avraham, *Jerusalem*
Yaron Bar-Dayan, *Holon*
Shomron Ben-Horin, *Tel-Hashomer*
Boris Kirshtein, *Beer Sheva*
Stephen Malnick, *Rehovot*
Yaakov Maor, *Tel-Hashomer*
Rifaat Safadi, *Jerusalem*
Nachum Vaisman, *Tel Aviv*

**Italy**

Rosaria Acquaviva, *Catania*
Alessandro Antonelli, *Pisa*
Giacosa Attilio, *Genova*
Salavtore Auricchio, *Naples*
Guido Basilisco, *Milan*
Antonio Basoli, *Rome*
Massimo Bellini, *Pisa*
Luigi Bonavina, *Milano*
Alfio Brogna, *Catania*
Giuseppe Calamita, *Bari*
Raffaele Capasso, *Naples*
Ignazio Castagliuolo, *Padova*
Francesco Cresi, *Torino*
Rosario Cuomo, *Napoli*
Salvatore Cuzzocrea, *Gazzi*
Mario M D'Elios, *Florence*
Cinzia Domeneghini, *Milan*
Luca Elli, *Milano*
Walter Fries, *Messina*
Eugenio Gaudio, *Rome*
Marco Gobbetti, *Bari*
Fabio Grizzi, *Rozzano*
Enzo Grossi, *Milanese*
Enzo Ierardi, *Foggia*
Pietro Invernizzi, *Monza*
Angelo A Izzo, *Naples*
Anna Kohn, *Rome*
Giovanni Latella, *L'Aquila*
Massimo Marignani, *Rome*
Sergio Morini, *Rome*
Raffaele Pezzilli, *Bologna*
Cristiano Rumio, *Milan*
Giovanni Sarnelli, *Naples*
Edoardo V Savarino, *Padua*
Pierpaolo Sileri, *Roma*
Annamaria Staiano, *Naples*
Giacomo C Sturniolo, *Padua*
Claudio Tiribelli, *Trieste*

**Japan**

Hirotada Akiho, *Fukuoka*
Akihiro Asakawa, *Kagoshima*
Hisashi Aso, *Sendai*
Yasu-Taka Azuma, *Osaka*
Shotaro Enomoto, *Wakayama*
Mikihiro Fujiya, *Hokkaido*
Takahisa Furuta, *Hamamatsu*
Akira Hokama, *Nishihara*
Ryota Hokari, *Saitama*
Yuichi Hori, *Hyogo*
Masahiro Iizuka, *Akita*
Shoji Ikuo, *Kobe*
Motohiro Imano, *Osaka*

Hajime Isomoto, *Nagasaki*
Tatehiro Kagawa, *Isehara*
Haruki Kitazawa, *Sendai*
Shigeru BH Ko, *Tokyo*
Xiao-Kang Li, *Tokyo*
Noriaki Manabe, *Okayama*
Atsushi Masamune, *Sendai*
Hiroyuki Matsubayashi, *Suntogun*
Kazuyuki Matsushita, *Inohana Chuo-ku*
Reiko Miyazawa, *Gunma*
Kazunari Murakami, *Oita*
Hikaru Nagahara, *Tokyo*
Yuji Naito, *Kyoto*
Atsushi Nakajima, *Yokohama*
Shoji Natsugoe, *Kagoshima City*
Tsutomu Nishida, *Suita*
Koji Nomoto, *Tokyo*
Shouji Shimoyama, *Tokyo*
Goshi Shiota, *Yonago*
Hidekazu Suzuki, *Tokyo*
Hitoshi Takagi, *Gunma*
Toru Takahashi, *Okayama*
Yoshihisa Takahashi, *Tokyo*
Kan Uchiyama, *Chiba*
Yoshiyuki Ueno, *Sendai*
Takato Ueno, *Fukuoka*
Hisayuki Uneyama, *Kwasaki*
Mitsunori Yamakawa, *Yamagata*
Takayuki Yamamoto, *Mie*
Yutaka Yata, *Maebashi-city*
Naohisa Yoshida, *Kyoto*
Hitoshi Yoshiji, *Kashihara*

**Lebanon**

Costantine F Daher, *Byblos*
Assaad M Soweid, *Beirut*
Julnar Usta, *Beirut*

**Malaysia**

Andrew CS Boon, *Ipoh*

**Mexico**

José M de la Roca-Chiapas, *Leon*
Maria-Raquel Huerta-Franco, *Leon*

**Netherland**

Wouter J de Jonge, *Amsterdam*
Aldo Grefhorst, *Groningen*
Daniel Keszthelyi, *Maastricht*
Cornelis FM Sier, *ZA Leiden*
Pieter J Tanis, *AZ Amsterdam*
Luc JW van der Laan, *Rotterdam*
Sander van der Marel, *Leiden*

**Norway**

Anne M Bakke, *Oslo*

**Poland**

Stanistaw A Hac, *Gdansk*
Stanislaw J Kowalski, *Krakow*
Agata Mulak, *Wroclaw*
Napoleon Waszkiewicz, *Choroszcz*

**Portugal**

Ricardo Marcos, *Porto*

**Romania**

Mihai Ciocirlan, *Bucharest*

**Russia**

Ludmila Filaretova, *Petersburg*

**Singapore**

Madhav Bhatia, *Singapore*
Brian K Goh, *Singapore*
Khek-Yu Ho, *Singapore*
Cliff KS Ong, *Singapore*

**South Korea**

Jae Hee Cheon, *Seoul*
Myung-Haing Cho, *Seoul*
Ki-Baik Hahm, *Seongnam*
HO Jae Han, *Gwangju*
Chang-Duk Jun, *Gwangju*
Sang Geon Kim, *Seoul*
Hong Joo Kim, *Seoul*
Jin-Kyung Kim, *Gyeongsan-Si*
Won-Jae Lee, *Seoul*
Sung-Joo Park, *Iksan*
Seung Ha Park, *Gangwon Do*
Kwan-Kyu Park, *Daegu*

**Spain**

Raquel Abalo, *Madrid*
Juan G Abraldes, *Barcelona*
Dario Acuna-Castroviejo, *Armilla*
Agustin Albillos, *Madrid*
Maria-Angeles Aller, *Madrid*
Fernando Azpiroz, *Barcelona*
Ramón Bataller, *Barcelona*
Marco Bustamante-Balén, *Valencia*
Andres Cardenas, *Barcelona*
Joan Claria, *Barcelona*
Pere Clavé, *Barcelona*
Manuel B de Acosta, *Santiago*
Manuel Giner, *Madrid*
Borja Hernandez-Breijo, *Madrid*
Angel I Lanas, *Zaragoza*
Maite T Martin, *Barcelona*
Vicente Martinez, *Bellaterra*
Jose M Mates, *Malaga*
Julio Mayol, *Madrid*
Marcal Pastor-Anglada, *Barcelona*
María E Sáez, *Seville*
Yolanda Sanz, *Burjassot*
Carlos Taxonera, *Madrid*
Maria D Yago, *Granada*

**Sweden**

Marco Del Chiaro, *Stockholm*
Frida Fak, *Gothenburg*
Gunnar FA Flemstrom, *Uppsala*
Evangelos Kalaitzakis, *Gothenburg*
Kristina Lamas, *Umea*
Bob R Olsson, *Goteborg*
Sara M Regnér, *Malmo*
Peter T Schmidt, *Stockholm*
Xiao-Feng Sun, *Linkoping*
Henrik Thorlacius, *Malmo*
Curt Tysk, *Orebro*

**Switzerland**

Jyrki J Eloranta, *Zurich*
Remy Meier, *Liestal*
Catherine M Pastor, *Geneva*

**Thailand**

Thawatchai Akaraviputh, *Bangkok*
Weekitt Kittisupamongkol, *Bangkok*

**Turkey**

Mehmet Bektas, *Ankara*
Ugur Duman, *Bursa*
Mukaddes Esrefoglu, *Istanbul*
Ahmet Guven, *Ankara*
Muammer Karadeniz, *Bornova-Izmir*
Elvan Ozbek, *Sakarya*
Ilhami Yuksel, *Ankara*

**Ukraine**

Oksana S Zayavhkivska, *Lviv*

**United Kingdom**

Geoffrey Burnstock, *London*
Janice E Drew, *Aberdeen*
Girish L Gupte, *Birmingham*
David C Hay, *Edinburgh*
Nusrat Husain, *Manchester*
Michael L Lucas, *Glasgow*
Jamie Murphy, *London*
Vadim Sumbayev, *Chatham Maritime*
Wing-Kin Syn, *Birmingham*
Andrea Varro, *Liverpool*

**United States**

Sami R Achem, *Florida*
Tauseef Ali, *Oklahoma City*
Shrikant Anant, *Oklahoma*
Mohammed S Anwer, *North Grafton*
Andrew Aronsohn, *Chicago*
Toms Augustin, *Sayre*

WJGP

World Journal of Gastrointestinal Pathophysiology

Contents

Quarterly Volume 8 Number 2 May 15, 2017

*World Journal of Gastrointestinal Pathophysiology*
**Volume 8 Number 2 May 15, 2017**

# Contents





NAME OF JOURNAL
*World Journal of Gastrointestinal Pathophysiology*

ISSN
ISSN 2150-5330 (online)

LAUNCH DATE
April 15, 2010

Frequency
Quarterly



EDITORIAL OFFICE
Xiu-Xia Song, Director
*World Journal of Gastrointestinal Pathophysiology*
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
May 15, 2017



INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Gastrointestinal Pathophysiology

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

*World J Gastrointest Pathophysiol* 2017 May 15; 8(2): 11-26

DOI: 10.4291/wjgp.v8.i2.11

ISSN 2150-5330 (online)

DIAGNOSTIC ADVANCES

# Nonalcoholic fatty liver disease: Diagnostic biomarkers

Fatemeh Hadizadeh, Elham Faghihimani, Peyman Adibi

Fatemeh Hadizadeh, Department of Biosciences and Nutrition, Karolinska Institute, 17177 Stockholm, Sweden

Elham Faghihimani, Peyman Adibi, Gastroenterology Research Center, Isfahan University of Medical Sciences, Isfahan 81746 73461, Iran

Author contributions: Adibi P had the original idea; Hadizadeh F and Faghihimani E performed the literature review and drafted the manuscript; Adibi P performed critical revision of the manuscript.

Conflict-of-interest statement: The authors declare no conflict of interest.



Manuscript source: Invited manuscript

Correspondence to: Elham Faghihimani, MD, Gastroenterology Research Center, Isfahan University of Medical Sciences, Hezar Jarib Blvd., Isfahan 81746 73461, Iran. efimani@yahoo.com
Telephone: +98-31-38023060
Fax: +98-31-36687898

Received: June 27, 2016
Peer-review started: June 29, 2016
First decision: August 5, 2016
Revised: January 10, 2017
Accepted: February 8, 2017
Article in press: February 13, 2017
Published online: May 15, 2017

## Abstract

Nonalcoholic fatty liver disease is a common medical condition worldwide and its prevalence has increased notably in the past few years due to the increases in prevalence of obesity and metabolic syndrome. However, diagnosis of this disease is still a matter of debate because of disease variations and pathophysiologic alterations. Specific single markers have gained considerable attention recently, among them markers related to hepatic pathophysiology, inflammation, adipocytokines and so forth. But, it seems that no single marker is sufficient for diagnosis and staging of the disease, and applying a panel including different types of tests may be more useful.

**Key words:** Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Liver fibrosis; Cirrhosis



**Core tip:** Nonalcoholic fatty liver disease is a pandemic disease in both developed and developing countries. There is emerging scientific evidence in this field that needs to be classified and summarized to make conceptual maps for researchers as well as practitioners. This article aimed to cover diagnostic markers in this disease, considering limitations and applications.

Hadizadeh F, Faghihimani E, Adibi P. Nonalcoholic fatty liver disease: Diagnostic biomarkers. *World J Gastrointest Pathophysiol* 2017; 8(2): 11-26 Available from: URL: http://www.wjgnet.com/2150-5330/full/v8/i2/11.htm DOI: http://dx.doi.org/10.4291/wjgp.v8.i2.11

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic hepatic diseases in many developed countries. It is also a serious health problem all over the world[1]. This disease includes a broad range of hepatic disorders from simple fat accumulation in hepatic cells (simple steatosis) to hepatic tissue

inflammation and fibrosis (steatohepatitis) and finally cirrhosis and even hepatocellular carcinoma[2-6]. The prevalence of this disease has considerably increased in the past years, and different studies have reported 2-fold increase in its prevalence within 10 years in many countries[7-9].

Diagnosis of NAFLD is of special importance because of the odds of its progressing to more critical stages. Furthermore, in cases of diagnosis at early stages, it is possible to prevent disease aggravation by applying simple approaches such as increasing physical activity and diet modification. Even in more advanced stages of the disease, such as non-alcoholic steatohepatitis (NASH), it is still important to diagnose it as early as possible due to its potential for progressing to cirrhosis[10]. In patients suspected of having cirrhosis, it is necessary to take other diagnostic measures into consideration, such as studying complications of portal hypertension (*e.g.,* screening for gasteroesophageal varicose veins) and examining the risk of hepatocellular carcinoma development[11-14]. So, it is important to be able to differentiate between simple steatosis and steatohepatitis as the beginning step of the progression towards severe and more progressive stages of the disease, such as fibrosis and cirrhosis[15,16].

At present, liver biopsy is the gold standard for diagnosing nonalcoholic fatty liver, but this method is not only invasive and expensive but also has the important limitations of pain, reluctance of patients, risk of severe complications and being subject to sampling error. All these challenges have increased motivation for finding/applying noninvasive methods for diagnosis of the different stages of NAFLD[17]. In this study, we provide a brief review of the individual main indices invented for the diagnosis and prediction of different stages of NAFLD.

## HEPATIC MARKERS

More than 60 transamination reactions have been recognized in liver, among which only the transaminases of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are of clinical value. Both of these markers, but especially ALT, are commonly used in clinical medicine as indicators of hepatic damage[18]. In many studies on patients suffering from NAFLD, increased levels of aminotransferases along with diabetes have been considered as independent predictors of moderate to severe fibrosis[19] in patients with fatty liver who are at risk of progression to advanced fibrosis and only these two variables have been shown to have a significant association with steatohepatitis[20].

### *ALT*

In many people, the asymptomatic increase of transaminases level, particularly ALT, has been observed, and the prevalence of this type of case (asymptomatic increase of ALT) has been reported differently in different populations (7.3% in the United States), 15.24% in Spain, and 21.7% in Scotland)[18,21], but it generally seems that it has a prevalence of 4% to 21.7%[18]. Of note, almost one-third of the people who have shown increased level of serum transaminases in their first test have demonstrated normal level of enzyme in the second one[18].

High waist circumference, high body mass index (BMI), male sex, alcohol consumption and young age are considered as strong predictors for rising ALT level[21]. Moreover, ALT level has been shown to have a positive association with the number of signs and symptoms of metabolic syndrome present[22]. In addition, serum ALT level has been shown to have a positive correlation with serum triglyceride, serum dehydroepiandrosterone sulfate (DHEAS), plasma fasting glucose and BMI, and a negative correlation with hemoglobin (Hb), serum high-density lipoprotein and age[23].

In some studies, NASH has been reported as the most prevalent cause of constant increases in serum ALT in asymptomatic blood donors[24] and it has been demonstrated that 32% of people who had ultrasound examination have been diagnosed with steatohepatitis accompanying asymptomatic increases of transaminases level[18]. In another study, intense fibrosis was shown to be independently predictable by ALT and two other indices (serum ferritin and diabetes)[25]. Other studies have also indicated a relationship between presence of steatohepatitis and increased level of ALT[26], and even ALT has been considered as a part of some steatohepatitis diagnostic panels, like the BAAT score[27], FIB4 index[28], FibroTest[29], FibroMeter[30], NashTest[29] and NFS[30].

In contrast, some studies have demonstrated that some patients suffering from NAFLD have normal serum level of ALT. In a study on a United States' population (the Dallas Heart Study), 79% of the adults with fatty liver had normal aminotransferase level[31], which was the same as the 55% of the Italian adults suffering from this disease in another study (the Dionysos Study)[32]. In a study of 2287 individuals from different tribes, almost one-third of the population had hepatic steatosis, among whom 79% showed normal ALT level[33]. In another study, normal serum transaminases level was reported in 46% of patients with steatohepatitis[34]. Of interest, a research study into the risk of NAFLD development in cases of normal serum levels of ALT (*i.e.,* lower than 35 U/L) indicated that the rise in serum ALT concentration - even within the normal range - was an independent predictor for NAFLD development[35]. In a study on diabetic patients, it was also shown that high-normal ALT could increase the risk of nonalcoholic fatty liver by 2.7-times[36].

There is no consensus on cutoff point and maximum normal concentration of ALT, and a broad value range, between 26 and 66 IU/L, has been suggested in different studies[37]. Some studies have shown that risk of NAFLD increases as ALT rises to more than 19 and 30 in women and men, respectively. Accordingly, these values have been suggested as maximum probable normal values in

these groups[38]. In a community-based study of 1346 Japanese patients with diagnostic criteria of NAFLD, the cutoff value of ALT for diagnosis of this disease was estimated to be 25 U/L in men and 17 U/L in women[39]. For the BAAT score, a normal range of ALT is considered between 1 and 19[27]. In another study that considered 19 and 30 (for women and men respectively) as the cutoff values for screening NASH among NAFLD patients, 99% and 8% sensitivity and specificity respectively were shown, while 40 U/L - which is in use in medicine - had 86% sensitivity and 32% specificity[26].

Thus, some researchers have rejected considering a reduction of ALT level (within reference range) as a tool for increasing its sensitivity because it leads to the adverse effect of labeling large numbers of asymptomatic individuals as patients[40]. Nevertheless, while some researchers believe that none of these values has enough sensitivity and specificity to support introduction of ALT as a reliable screening test for diagnosing steatohepatitis among patients with fatty liver[26,41]. Some other researches have suggested this enzyme as the best individual marker for detecting fat infiltration in the liver[42].

Riquelme *et al*[43] showed that, in a Hispanic population, ALT greater than 14 had an odds ratio (OR) equal to 13.95 for fatty liver. Moreover, although some studies have mentioned that this enzyme cannot be considered as an index for prediction/diagnosis of steatosis or fibrosis[44], in a study conducted on patients with type 2 diabetes, ALT was the only variable independently related to steatohepatitis in these patients[45]. In another study, ALT was found to be considerably higher in patients with steatohepatitis, even when within normal ranges[45]. And, a study on obese patients showed that elevated ALT level had a significant correlation with incidence of steatohepatitis and fibrosis[34,46]. In contrast, a study conducted on 73 untreated patients - to investigate the relationship between changes in hepatic enzymes and histological liver changes in NAFLD - indicated absence of a clear relationship between the pattern of ALT levels and changes in steatosis, inflammation, hepatocyte ballooning or degree of fibrosis over time. Results of this study also demonstrated the level of hepatic enzymes as an insensitive tool for following histological changes of liver among patients with NAFLD[47]. Regarding more advance stages of the disease, Kaneda *et al*[48] showed ALT level to be significantly different in patients with cirrhosis, compared to patients with earlier stages (50 IU/L *vs* 87 IU/L).

### AST

AST is another hepatic transaminase that plays a role in diagnosis of steatohepatitis. Up to 3.6% of people in the United States have asymptomatic increase in AST[21], and it seems that this hepatic index is related to metabolic syndrome and BMI[49]. In Asian studies, AST is considered as an independent marker for severity of hepatic fibrosis if it is at least twice as much as the maximum normal value[50]. This marker has been used in different diagnostic panels, such as the FibroTest[12,29,51], NashTest[52], NAFLD Fibrosis Score (NFS)[53,54], FibroMeter[55], FIB4 index[28] and other different models[56,57]. In addition, two common tests of AST to ALT ratio and the AST to platelet ratio index (APRI) have been generated using this enzyme.

The AST to ALT ratio, which is frequently used in medicine, is known as an independent index for predicting presence of advanced hepatic fibrosis and has been used as a part of different panels, such as the NAFLD Fibrosis Score[26], ScoreBARD[58] and other panels[28,41,57]. The APRI is another non-invasive index for studying hepatic fibrosis in patients with NAFLD and it has been reported as preferred over the AST to ALT ratio for predicting advanced hepatic fibrosis[59].

### Alkaline phosphatase

Higher level of alkaline phosphatase (ALKP) can also be considered as a marker relating to hepatic fibrosis in patients with steatohepatitis. In a study conducted on NASH patients to investigate the association between serum levels of ALKP with the degree of hepatic fibrosis, it was shown that serum level of this enzyme was significantly higher in patients suffering from NASH as compared to those without it[60]. Another study represented ALKP to be significantly higher in NASH patients, even when within normal range[45]. In addition, the increased level of ALKP has been reported as an independent predictor for hepatic diseases-related death[61].

### Albumin

In different studies albumin has been identified as a factor related to steatohepatitis, septal and intensive fibrosis[27,48,62] and cirrhosis[48] and as an independent predictor of hepatic-related mortality[61,63]. In a study on 73 untreated NAFLD patients, a significant decrease was reported in serum albumin level of patients during 2-year follow-up (from 0.5 ± 4.3 at the beginning of the study to 0.4 ± 4.2 after 24 mo)[47]. In another study, serum level of this protein showed OR of 0.049 (95%CI: 0.003-0.879) and of 0.057 (95%CI: 0.007-0.477) for prediction of steatohepatitis and severe fibrosis, respectively[62].

In an investigation by Suzuki *et al*[64], serum albumin was reported significantly lower in patients with intensive hepatic fibrosis (stage 3-4) compared to those in earlier stages (4.1 ± 0.09 *vs* 4.4 ± 0.05). In another study, serum level of albumin was reported as 4.4 g/dL (range: 3.5-5.4 g/dL) in patients with mild fibrosis and 4.2 g/dL (range: 4.6-2.6 g/dL) in patients with intensive fibrosis, and the difference was statistically significant[48]. In the same study, the serum level of this protein showed a significant difference in patients with and without cirrhosis (4 g/dL *vs* 4.4 g/dL)[48]. However, in a study by Sumida *et al*[65], although concentration of serum albumin showed diminishment when the

intensity of NAFLD increased, this decreasing trend was not statistically significant (4.51 g/dL in simple steatosis, 4.37 g/dL in mild steatosis and 4.29 g/dL in advanced steatosis).

## INFLAMMATORY MARKERS

### *Platelets*

Thrombocytopenia is a common finding in advanced stages of all chronic hepatic diseases, and platelet count is clinically important to predict status of hepatic fibrosis in patients with chronic hepatic diseases[66]. In NAFLD, platelets are important in terms of absolute number as well as volume. In different studies, reduction of platelets to fewer than 160000 has been considered as an independent marker for severity of hepatic fibrosis[17,50].

In a study by Suzuki *et al*[64], platelet count was reportedly significantly lower in patients with intensive hepatic fibrosis (stage 3-4), compared to patients in earlier stages (180 ± 17 $10^3$/mm$^3$ *vs* 247 ± 10 $10^3$/mm$^3$). The result of this study has been confirmed by other studies[48]. In a study in 2006, when the cutoff point of 16 × $10^4$/μL was used, platelet count was shown to have 100% sensitivity, 95% specificity, 76% positive predictive value (PPV) and 100% negative predictive value (NPV) for diagnosis of cirrhosis[48]. In this study, platelet count was determined as an independent predictor among different markers and it was shown that this index significantly decreased in cases of cirrhosis (from 241 × $10^3$/μL to 130 × $10^3$/μL)[48]. In addition, in another study conducted on NAFLD patients from 9 hepatology centers in Japan, it was shown that platelet count linearly decreased with the increase of histological intensity of hepatic fibrosis. The cutoff value of 19.2 × $10^4$ was introduced and shown to provide a sensitivity of 62.7% and a specificity of 76.3% for diagnosis of the 3$^{rd}$ stage of hepatic fibrosis, while 15.3 × $10^4$ was selected as the optimal cutoff value for diagnosis of the last stage of this disease (sensitivity: 80.5%, specificity: 88.8%)[67].

Mean platelet volume (MPV) is considered as an indicator of platelet function[68]. In 2010, Ozhan *et al*[69] showed that patients with NAFLD have higher MPV than individuals without it (10.43 ± 1.14 *vs* 9.09 ± 1.25). This study also showed that MPV had a positive correlation with AST and ALT levels, and a negative correlation with platelet count. These results have been confirmed in another study conducted on obese patients which showed a significant association between NASH and MPV, and which indicated an increase in the prevalence of NASH by increasing the values of MPV after adjustment for all the confounding variables[70].

In contrast, another study with 60 NAFLD cases and 54 healthy controls, the MPV did not show any difference between the two groups. This study also concluded that in the absence of other MPV metabolic risk factors, MPV does not play a role in the mechanism of increasing the risk of cardiovascular diseases in patients with fatty liver[71]. Moreover, in another study in 2012, which was conducted to investigate the relationship between MPV index and NAFLD, although MPV was increased in patients with NAFLD, no association was detected between degree of steatosis, lobular hepatitis, hepatocellular ballooning, NAFLD activity score and fibrosis with the values of MPV. In this study, it did not show a correlation with increase of resistance to insulin[68].

Considering the novelty of this hypothesis (significant association between MPV and NAFLD) and remembering the scarcity of the studies conducted to examine this hypothesis so far, it seems that there is still need for more studies to make a clear conclusion. Furthermore, considering that platelets usually decrease in stage 4, it can be probably concluded that a decrease in platelets usually indicates the occurrence of more advanced stages of fibrosis, which is usually equivalent to cirrhosis[70]. Since platelet count is a simple, cost-effective and accurate method, it can be considered as a suitable biomarker for diagnosis of fibrosis severity in these patients[72].

### *C-reactive protein*

C-reactive protein (CRP) is an acute phase reactant, which has many applications in clinic[73]. It seems that serum concentration of CRP is a strong index for predicting the incidence of NAFLD[74] and some studies have introduced increased serum level of CRP as an independent risk factor for development of NAFLD[74,75]. In addition to routine measurement of CRP, another method has been invented to measure values of high-sensitivity CRP (hs-CRP). This method promises to make detection of even low degrees of inflammation possible[76]. In some studies, hs-CRP has been called a diagnostic tool, which not only can be effective in differentiation of steatohepatitis but also can specify the severity degree of hepatic fibrosis in patients with NASH and is probably able to differentiate between advanced and mild fibrosis among these patients[65,76]. This ability has been mentioned to persist even after adjusting for the effect of different confounding variables, such of age, sex, diabetes, dyslipidemia, BMI, subcutaneous fat and intra-abdominal visceral fat[76]. Another research study has shown that hs-CRP level was higher in patients with more severe grades of steatohepatitis (grades 2 and 3), rather than in patients with mild or simple grades of NASH[77].

Although several studies have shown a relationship between NAFLD and serum concentration of hs-CRP, results of some studies are in contrast to this hypothesis[74]. It was concluded in one study that hs-CRP could be an index for determining steatosis in obese patients, but there is no association between this marker and NASH[78]. In parallel, a cohort study could not find any relationship between hs-CRP level and severity of hepatic steatosis. The result of this study indicated that hs-CRP is not helpful in predicting histological intensity of NAFLD[73]. Of note, some investigations

have also demonstrated increased serum levels of hs-CRP in disorders related to NAFLD, such as obesity, insulin resistance and manifestations of metabolic syndrome[73,78-82]; although, there is doubt about the presence of a causal relationship between this marker and metabolic syndrome[79].

CRP is mostly produced in liver, but it seems that it is also produced in the adipose tissue[80]. Furthermore, adipose tissue can act as an endocrine organ and secretes some inflammatory cytokines, like interleukin (IL)-6, leading to stimulation of the liver for CRP production[83]. So, generally, it seems that obesity is one of the strongest determinants of serum level of CRP. Of interest, serum level of CRP is higher in women than in men[83-85], and the results of one study have shown that an increase in serum level of hs-CRP is significantly associated with intensity of NAFLD, only in women[74]. One explanation for this could be related to the sex difference in correlation between CRP and obesity, which in turn can be justified by the difference between the two sexes in terms of amount and pattern of adipose tissue distribution[83].

One of the disadvantages of this index is the effect of different factors, such as race, age, sex, smoking or alcohol consumption, on its serum concentration[74,86,87]. On the other hand, it seems that an accurate and accepted cutoff point has not yet been determined for this marker. Median CRP value of about 1 has been introduced for diagnosis of steatohepatitis, while the suggested cutoff value for hs-CRP for diagnosis of metabolic syndrome, NASH and prediction of the risk of cardiovascular complications is 0.65 mg/L[88].

In sum, considering most studies conducted in this field, it seems that this index can be regarded as a promising biomarker for screening steatohepatitis in the future[77].

### *Iron (ferritin)*

Iron is considered as an element that reacts to oxygen radicals. High rates of blood ferritin and increased iron accumulation in liver have been reported in steatohepatitis, which can be attributed to systemic inflammation, increase in iron storage, or both[89]. Ferritin level usually increases in 20%-50% and transferrin saturation increases in 5%-10% of NAFLD patients[90]. One study showed that elevation of serum ferritin level by 1.5-times as much as the maximum normal rate was related to accumulation of iron in liver, diagnosis of steatohepatitis and worsening of histological activity of this disease. It has been introduced as an independent index for diagnosis of advanced hepatic fibrosis among patients with fatty liver, which can probably be applied as a useful index for identifying the patients who are susceptible to steatohepatitis and fibrosis[89].

In the NAFIC scoring method, serum ferritin levels of equal or more than 200 ng/mL and 300 ng/mL in women and men, respectively, have been used as an independent variable for diagnosis of NASH[91]. Of note, although the study by Sumida *et al*[91] showed an increase in concentration of ferritin by increasing the severity of NAFLD, the difference was not statistically significant (179 ng/mL in simple steatosis, 241 ng/mL in mild steatohepatitis and 278.1 ng/mL in advanced steatohepatitis). On the other hand, some researchers believe that hemocystein level in serum may be able to independently predict steatohepatitis and could be applied as another noninvasive marker for evaluating NAFLD. Hemocystein level of serum in NAFLD is not different from that in healthy people, while it is considerably decreased in patients with steatohepatitis[92].

### *Malondialdehyde*

In the two-hit theory - one of the most acceptable theories to justify the progression of NAFLD to NASH - an oxidative stress, which may lead to lipid peroxidation, is considered as the most probable mechanism for the second hit. During the process of lipid peroxidation, a wide range of pre-inflammatory and fibrogen products are produced as by-products, which result in progression of the disease. One of these by-products is Malondialdehyde (MDA)[93]. MDA can stimulate hepatic stellate cells and result in fibrosis by producing collagen[94].

In a study from 2010, MDA with a cutoff point of 11 had sensitivity of 60%, specificity of 92%, PPV of 81%, NPV of 81% and positive likelihood ratio (+LR) of 7.9 for predicting the presence of NASH[65]. Another study which investigated the relationship between MDA level as index for oxidative stress, antioxidant vitamins A and alpha-tocopherol with presence of steatohepatitis showed that level of vitamin A and MDA increased in simple steatosis and steatohepatitis and alpha-tocopherol level significantly decreased in patients with steatosis and steatohepatitis compared with healthy people. In this study, although there was significant difference between patients with steatosis and steatohepatitis, the authors concluded that none of these indices had relationship with histopathologic severity of the disease[93].

### *Plasma pentraxin 3*

Pentraxin is a family of proteins divided into two short and long classes, based on the length of their structure. CRP and serum amyloid P are two short parts of this family. Plasma pentraxin 3 (PTX3) is one of the long proteins of this family, and it is significantly higher in patients with steatohepatitis than those without. Serum concentration of this protein is higher in patients suffering from more advanced stages of NAFLD and higher values of this protein are correlated with higher stages of the hepatic fibrosis. So, it seems that serum PTX3 level could be used as a marker for diagnosis of the severity of hepatic fibrosis, in addition to differentiating between steatohepatitis and simple steatosis[95].

### *Adipocytokines*

Adipocytokines are a large family of proteins produced

and secreted by adipose tissue and which have close relationship with the inflammation process[87]. Adipokines and cytokines play a major role in regulation and orchestration of inflammatory processes all over the body and play an important role in insulin resistance pathogenesis and NAFLD through complex and mutual paracrine and endocrine mechanisms. Some adipocytokines reduce insulin resistance, like adiponectin and leptin, while others lead to increased insulin resistance, like IL-6 and tumor necrosis factor-alpha (TNF-α)[96].

Some studies have shown that the concentration of serum adipokines in human can be used as an index for diagnosis of NAFLD, especially in its advanced stages[46]; however, other studies have not been able to find a significant association between adipokines and histopathological intensity of this disease[97] and it has been concluded that adipokines cannot differentiate between benign and advanced histological stages of NAFLD[46]. It seems that there is a complex relationship between adipocytokines and pathogenesis of NAFLD[98], and the balance between proinflammatory and anti-inflammatory function of adipocytokines may play an important role in the development of this disease[99].

### *Adiponectin*

Adiponectin is a collagen-like protein derived from adipocytes with anti-inflammatory and anti-lipogenic effects[100]. This adipokine, which is circulating abundantly in human serum, protects against excessive accumulation of fat in the liver and subsequently protects liver against inflammation and fibrosis. Serum level of adiponectin decreases in obese people and shows greater decrease in patients with steatosis and steatohepatitis[7,46,101], being higher in patients with lower degrees of steatosis[102]. Hypoadiponectinemia has been demonstrated to be a predictive factor for necro-inflammation and more severe grades of fibrosis, even after exclusion of the effect of variables such as age, BMI and waist circumference[103]. It is among the variables which have a direct relationship with steatohepatitis as compared to simple steatosis[98].

A study designed to measure liver fat, intraabdominal fat and subcutaneous fat, along with insulin resistance indices and adiponectin, showed that serum adiponectin had a reverse relationship with hepatic fat content[9]. Hypoadiponectinemia is accompanied by increased risk of cardiovascular diseases and it seems to be a key factor in metabolic syndrome[102], although some studies have shown that hypoadiponectinemia in steatohepatitis is independent from insulin resistance[73,100]. It seems that hypoadiponectinemia is a primary finding in steatohepatitis, which is identifiable from a very long time before the appearance of diabetes or emergence of central obesity and which has a correlation with histological intensity of hepatic damage.

The serum level of adiponectin was shown to be significantly lower in patients with steatohepatitis than in a control group (344 ± 5.476 *vs* 836 ± 11.548), while no difference was detected between the two groups in terms of other cytokines[103]. In another study, the serum level of adiponectin was found to be significantly lower in patients with primary stages of steatohepatitis, compared to patients with simple steatosis (3.6 mug/mL *vs* 6.0 mug/mL). In this study, adiponectin showed higher differentiation power than serum level of type Ⅳ collagen 7s and the homeostasis model assessment of insulin resistance (HOMA-IR) and it had sensitivity of 68% and specificity of 79% for predicting primary stages of steatohepatitis[104].

Considering the association between single nucleotide polymorphisms (SNPs) of the adiponectin gene and insulin resistance and increase of prevalence of type 2 diabetes, a study was conducted to determine the association between variations in this gene and NAFLD development, which indicated a positive association, especially with hepatic fibrosis[105]. Another study showed a considerable decrease in the mRNA expression of adiponectin and RⅡ receptors (adipoRⅡ) in the liver of NASH patients as compared to patients with simple steatosis, which might be indicative for its pathophysiological relationship with NAFLD[106].

Adiponectin is a powerful anti-inflammatory adipocytokine for neutralizing TNF-α[99]. Lack of a relationship between circulating levels of adiponectin and its hepatic expression, shown in the study by Kaser *et al*[106] was interpreted such that hepatic expression of adiponectin was probably regulated by different factors, such as TNF-α. Owing to the importance of adiponectin as a diagnostic marker, it has been used in different noninvasive panels for diagnosis of NAFLD[104,107].

### *Leptin*

Leptin is a 16 kD non-glycosylated protein which is usually secreted from adipocytes of white adipose tissue[102,108]. Also, low amounts of leptin has been shown to be secreted by other tissues, such as placenta, skeletal muscles, stomach fundus and culture-activated hepatic stellate cells[108]. Leptin plays the role of a peptide hormone, regulating food uptake and energy consumption of the body through central feedback mechanisms and relating eating to hypothalamus and adipose tissue mass (*i.e.,* it controls food uptake and increases energy consumption)[102,108,109]. The importance of this regulatory role is such that recombinant leptin has been studied for treatment of all prevalent types of obesity in different clinical trials[110-113].

Some researchers believe that serum leptin level is associated with NAFLD. It has been shown that increased serum leptin level in patients with steatohepatitis has no relation with BMI of these patients, and this increase could not be easily justified by the patient's sex, obesity or type 2 diabetes[109,114]. In contrast, another study indicated a correlation between human serum level of leptin and body fat percent and BMI[115]. A study which was conducted on young adults (18-year-old to 21-year-

old students) to determine the risk factors of NAFLD in this age group showed serum level of leptin to be associated with abdominal wall fat index (AFI) (as the only independent risk factor of fatty liver in this study) in women, while it had no correlation with AFI in men[116].

Some studies have indicated a role for leptin in resistance of liver against insulin[115], and some others have connected leptin to atherosclerosis and cardiovascular diseases in obese patients[102]. However, a study designed to investigate the potential association between leptin with insulin resistance and histological changes in NAFLD patients was unsuccessful to show any association between serum level of leptin with fasting insulin level and severity of hepatic histological changes[117]. Another study conducted in 2003 showed increase in the serum level of leptin as one of the presentations of patients with steatosis and normal serum level of transaminases. In this study, a negative correlation was detected between serum leptin and serum transaminase levels, as well as progression of hepatocytes damage[118]. Another study which followed patients for 6 mo showed considerable decrease in the level of serum transaminase only in NASH patients with increased levels of serum leptin. This study concluded that leptin has a preventive effect on progression of hepatic damage in NAFLD[117].

Different studies have been conducted so far on factors relating to leptin and its role in the pathogenesis of NAFLD[119]. Some studies have shown that serum leptin level is directly associated with severity of hepatic steatosis and that increased levels of serum leptin may lead to increase of hepatic steatosis and steatohepatitis, but no association with emergence of inflammation and fibrosis was found[109,114]. Some other studies, however, have failed to show a significant association between serum level of leptin and steatosis[120] or steatohepatitis[46]. Still other studies have concluded that leptin could be used for diagnosis of hepatic fibrosis, but not for staging of this disease[121].

### *TNF-α*

TNF-α plays roles in the progression of NAFLD through different mechanisms. TNF-α has pro-inflammatory effects and activates harmful pathogenic routes by decreasing HDL-cholesterol, increasing expression of cholesterogenic genes and suppressing cholesterol exclusion[122]. It also stimulates synthesis of hepatic fatty acids, increases serum level of triglyceride[123] and decreases sensitivity to insulin[100,124]. In addition, TNF-α can also induce apoptosis in and proliferation of hepatic cells and play a role in pathogenesis of hepatic fibrosis[124]. The importance of TNF-α in emergence of fatty liver disease (with genetic or nutritional origin) has been demonstrated in different studies, and it has been shown that neutralization of TNF-α activity leads to improvement of insulin resistance and fatty liver disease[111].

Different studies have demonstrated that plasma level of TNF-α is higher in patients with NAFLD and NASH, compared to control groups[73,98,125-128], significantly or at least borderline significantly ($P$ = 0.052)[129]. In a study by Jarrar *et al*[98], TNF-α was introduced as the only independent predictor of fibrosis in patients with steatohepatitis. In another study conducted in 2005 to examine TNF-α as a potential noninvasive marker for studying histopathological intensity of NASH, serum level of TNF-α was higher in patients with steatohepatitis and cirrhosis than in the control group, but it did not show a significant association with histopathological severity of the disease[126]. Another confirmatory study by Hui *et al*[100] showed that TNF-α level was higher in patients with fatty liver and steatohepatitis as compared to healthy individuals, but it was not efficient for differentiating steatohepatitis from fatty liver.

A study in 2010 conducted on NAFLD children, showed that serum concentration of TNF-α and its soluble receptors were significantly higher in obese children with fatty liver than those in the control group. But, again, the abilities of this cytokine and its receptors were insufficient in differentiating between different grades of steatosis. In this study, this marker was introduced as a suitable serum marker for predicting hepatic steatosis in obese children[127]. However, another study conducted in 2005 did not find a significant difference between TNF-α level of patients with steatohepatitis as compared to the control group[103].

Of note, TNF-α usually increases in visceral obesity and NAFLD, and it has been shown that this cytokine plays a role in regulation of the body's iron homeostasis. A study in 2008 which investigated these associations showed that the highest serum concentration of this cytokine was found in NAFLD patients with iron overload. It has also been shown that phlebotomy treatment can reduce concentration of TNF-α in addition to level of ferritin and transferrin saturation and can lead to improvements in hepatic function tests. This study concluded that TNF-α plays a regulatory role for iron that leads to accumulation of ion in the liver of patients with NAFLD[125].

### *IL-6*

IL-6 is a multifunctional cytokine which regulates immune responses, the acute phase reaction and homeostasis[124] and is secreted from different cells in body, one of which is the adipocytes[83,100]. This cytokine causes stimulation of liver and some acute phase proteins, like PTX3 and CRP, are then produced in liver in response to it[129,130]. Serum level of IL-6 has considerable correlation with insulin-resistance, like TNF-α, which leads to increased resistance to insulin[99,100]. Also, IL-6 stimulates hepatic lipogenesis related to obesity and insulin-resistance[125]. It seems that this cytokine plays a critical role in pathophysiology of different aspects of NAFLD in humans[99].

Zamora-Valdés *et al*[131] showed in 2007 that chronic alternating hypoxia resulting from obstructive

sleep apnea, as one of the risk factors for NAFLD, led to increased serum level of IL-6. IL-6 level has also been shown to be significantly different between NAFLD patients and non-afflicted people in some studies[98]. Another study indicated a considerable increase in the serum level of TNF and IL-6 as well as of their soluble receptors in steatohepatitis, compared to simple steatosis, but no correlation was found between circulating levels of these cytokines and their receptors with either the degree of disease activity or fibrosis stage[132]. However, another study did not find any increase in the hepatic mRNA transcription and expression of IL-6 in patients with steatohepatitis as compared to patients with simple steatosis[76].

## STRUCTURAL MARKERS

### *Cytokeratin-18*

Different studies have indicated the key role of apoptosis in hepatic damage occurring in advanced stages of NAFLD (steatohepatitis) and, subsequently, use of hepatocyte apoptosis markers was suggested for diagnosis and staging of this disease[133-135]. During the apoptosis process, cytokeratin-18 (CK-18) is fragmented by caspase 3[135] and hepatocytes which leads to cell death through the apoptosis process and release fragments of CK-18 into the blood stream, so that the blood level of the fragmented CK-18 is associated with the presence of hepatic fibrosis[133]. Indeed, this substance has been shown to be significantly higher in patients with NASH[120].

Plasma CK-18 fragments have been studied and evaluated in large studies[72,136,137]. In one study, for diagnosis of NASH, blood level of more than 395 U/L of cytokeratin had sensitivity, specificity, PPV and NPV values of 85.7, 99.9, 99.9 and 85.7, respectively[134]. Younossi *et al*[107] reported sensitivity, specificity, PPV and NPV of cleaved CK-18 as 63.64, 87.23, 70 and 83.7, respectively, for the cutoff value of 174.1; however, when the cutoff point was increased to 261.35, specificity and PPV increased to 97.87 and 88.9, and when the cutoff decreased to 111.6, sensitivity and NPV changed to 81.82 and 77.8, respectively. In another study, sensitivity and specificity of this index were reported to be 78% and 87%, respectively, for diagnosis of steatohepatitis[138].

In one study conducted to investigate the clinical utility of different serum markers such as CK-18, hyaluronic acid and tissue inhibitor of metalloproteinase 1 (TIMP1) for the diagnosis of steatohepatitis, CK-18 was the only biomarker which could be helpful, having PPV of 81% and NPV of 85%[139]. In addition to the cleaved form of CK-18, its intact form, and also the difference between these two types (cleaved type and intact type), may play a role in evaluation of steatohepatitis. It has been reported that the serum level of intact CK-18 has higher predictive value than its cleaved form for the diagnosis of this disease. In the same study, intact CK-18, considering a cutoff point of 384.3, had sensitivity, specificity, PPV and NPV of 63.64, 89.36, 73.7 and 84.0, respectively; however, when the cutoff was increased to 545, its specificity and PPV increased to 95.74 and 80, respectively, and when the cutoff was decreased to 242.9, its sensitivity and NPV changed to 86.36 and 91.2, respectively[107].

Considering cleaved CK-18 as an apoptosis marker, intact CK-18 as a total apoptosis marker and cleaved CK-18-intact CK-18 as a necrosis marker[56], it seems logical that patients with steatohepatitis have higher levels of all of these 3 markers. Furthermore, it has been suggested that tissue polypeptide-specific antigen may be a serologic mirror for CK-18, so that it may also be also considered as an index for diagnosis of NASH (sensitivity, specificity, PPV and NPV of 95, 95.8, 22 and 0.09, respectively, with the cutoff value of 88 ng/mL)[135].

In sum, considering the validation of use of plasma CK-18 fragment in different studies, it can be claimed that all pieces of evidence have favored the usefulness of this index to differentiate between steatohepatitis and simple steatosis[140,141]. Therefore, some researchers believe that this biomarker can be applied as an ideal index for noninvasive diagnosis of steatohepatitis in the future, although it is not routinely accessible as a common laboratory test presently[72].

### *Hyaluronic acid*

Hyaluronic acid is a component of extracellular matrix that is produced by mesenchymal cells[48]. Its serum level in hepatic diseases is affected by production of collagen in liver and reduction of endothelial function of hepatic sinusoids[64]. It has been shown that serum level of hyaluronic acid is significantly higher in patients with steatohepatitis, compared to healthy controls, and that it is also higher in cirrhotic patients than in NASH patients[127].

Another study conducted to investigate the possibility of using hyaluronic acid for diagnosis of steatohepatitis reported an association between this marker and stage of NAFLD[140]. Yet another study has reported that cutoff value of greater or equal to 43 has sensitivity, specificity, PPV and NPV of 65.7%, 90.5%, 92% and 61.3%, respectively, for diagnosis of NASH[142]. Moreover, hyaluronic acid has been introduced in different studies as a good and reliable index for diagnosis of hepatic fibrosis[121,143,144]. A study which was conducted to investigate accuracy of serum hyaluronic acid in prediction of hepatic fibrosis intensity in NAFLD patients showed that this marker had a positive correlation with fibrosis degree. In this study, the cutoff point of 46.1 mg/L for serum hyaluronic acid demonstrated sensitivity, specificity, PPV and NPV of 85%, 80%, 51% and 96%, respectively, for diagnosis of fibrosis, and it was concluded that measurement of serum hyaluronic acid could be a useful tool for diagnosis of patients with severe fibrosis. Besides, the logarithm of hyaluronic acid in NAFLD patients was shown to be associated with the degree of hepatic fibrosis, age and serum albumin, and it was calculated as follows: [40.1 + 0.333 × (degrees of hepatic fibrosis) + 0.032 × (age)

- 0.561 × (serum albumin)].

Based on this model, an increase of fibrosis by one degree is accompanied by an increase of serum level of hyaluronic acid by 40%[64]. In another study, hyaluronic acid was demonstrated to have NPV of 100% for the diagnosis of patients without fibrosis, when considering a cutoff value of 42 ng/mL. Sensitivity, specificity and PPV of this marker were calculated as 100%, 89% and 77%, respectively[48]. Also, another investigation in this field has suggested hyaluronic acid and type Ⅳ collagen 7s as two independent predictors of severe fibrosis, with both capable of reflecting the degree and severity of hepatic fibrosis. In this study, the mean serum level of hyaluronic acid in patients with mild fibrosis was reported as 22 ng/mL, while it was 118 ng/mL in patients with severe fibrosis, and there was statistically significance[48].

In another study by Palekar *et al*[57], hyaluronic acid of more than 45.3 mcg/L was introduced as a good index for predicting advanced hepatic fibrosis and was regarded as the strongest independent predictor of severe fibrosis among some variables such as age, sex, AST, BMI and AST/ALT ratio[57,145]. In addition, because hyaluronic acid increases in stages 3 and 4 of hepatic fibrosis, compared to markers like platelet which usually only decreases in stage 4, hyaluronic acid can be applied for prediction of fibrosis in earlier stages[72]. Owing to its importance, it has been used in different diagnostic panels[13,143,146].

In contrast, in a study investigating the clinical application of this serum marker in diagnosis of steatohepatitis, hyaluronic acid was not approved to be suitable for diagnosis of this disease[140]. In another study, this index did not show a correlation with degree of fibrosis, compared to age, TNF-α and serum concentration of type Ⅳ collagen, which had a weak but significant association; although, it could probably be used to differentiate between mild (degrees 1 and 2) and advanced fibrosis[147].

In sum, it seems that serum level of hyaluronic acid is independently and significantly related to the presence of steatohepatitis and severe fibrosis[143], and it probably can be used as a suitable marker for monitoring the progression of fibrosis toward cirrhosis in NAFLD patients[126].

### *Collagen 7s*

Collagen 7s is another diagnostic marker that has been evaluated in different studies. Sakugawa *et al*[143] conducted research on the utility of the fibrosis marker of type Ⅵ collagen 7s for the diagnosis of steatohepatitis and reported an association between this index and the stage of fatty liver. To our knowledge, this is the only study conducted on type Ⅵ collagen 7s that has been referred to in different publications[141,148]. This marker has positive predictive value of 86% and 68.4%, negative predictive value of 61.8% and 83.6%, sensitivity of 70% and 81.3% and specificity of 81% and 71.4%, respectively, for diagnosing steatohepatitis and severe fibrosis when the cutoff point of ⩾ 5 ng/mL is considered.

Multiple studies have focused on type Ⅳ collagen 7s[76,98,112,149]. A strong and stable association between type Ⅳ collagen 7s and advanced fibrosis (before progress to cirrhosis) has been shown in different studies of patients with steatohepatitis[94]. A study by Yoneda *et al*[94] in 2007 showed that liver stiffness values had a suitable relationship with stage of hepatic fibrosis in patients with NAFLD and liver stiffness had a correlation with serum level of type Ⅳ collagen 7s. Type Ⅳ collagen 7s has been applied in different panels, such as NAFIC[98,104].

It seems that this marker is independently and significantly related to the presence of steatohepatitis and severe fibrosis and can be useful for differentiating between mild and severe forms of NAFLD[76,143].

## OTHER MARKERS

### *Dehydroepiandrosterone*

Dehydroepiandrosterone (DHEA) is one of the adrenal hormones with anti-oxidative stress effects that reduces resistance to insulin and is effective in mediating the expression of peroxisome proliferator-activated receptor alpha and mRNA of pro-collagen[149,150]. This hormone represent the most abundant steroid hormone in the body, and its interchangeable sulfated form (DHEA-S) has different functions, and influences on obesity, diabetes, atherosclerosis and osteoporosis[1,23,94].

Some studies have shown that body concentration of DHEA-S is independently and reversely correlated with death (resulting from all causes)[1]. It has also been shown that serum levels of DHEA-S have positive correlation with Hb, platelet, ALT, cholinesterase, albumin and triglyceride, and have negative correlation with age, AST, AST/ALT ratio, ALP, HDL-cholesterol, hyaluronic acid and type Ⅳ collagen 7s[65,149,150]. Thus, some researchers believe that DHEA may play a role in the pathophysiology of NAFLD and also in its progression toward more advanced stages of the disease *via* different mechanisms[23].

A study on menopausal women by Saruç *et al*[151] showed that women suffering from NAFLD had higher levels of DHEA and DHEAS than the control group (menopausal women with normal histology). In this study, DHEA and DHEAS showed positive correlation with BMI and waist circumference. A cross-sectional study conducted in 2010 on 1912 men demonstrated that steatohepatitis was associated with higher levels of serum DHEA-S, and the highest risk of NASH development was reported for those who had the highest serum level of DHEA-S (OR = 1.59, 95%CI: 1.04-2.43)[152]. Subsequently, a study conducted to investigate the clinical significance of serum level of DHEA-S in NAFLD patients showed that the serum level of this hormone was significantly higher in patients than in controls[23].

The study by Sumida *et al*[65] reported the same results, although the difference did not reach statistical significance (128.7 μg/dL in NAFLD patients *vs* 113.6 μg/dL in control group). However, this study demonstrated that patients suffering from severe stages of NAFLD (steatohepatitis with fibrosis of stage 3 or 4) had lower serum level of DHEA-S than patients with mild stages of the disease (simple steatosis or steatohepatitis with fibrosis of stage 0-2)[65].

In parallel, some other studies have also shown that NASH with advanced fibrosis is strongly associated with low concentration of circulating DHEA-S[149,153]. This association persisted even after adjustment for variables such as age, sex and resistance to insulin[65]. By applying single-variable analysis of serum level of DHEA in another study, a significant difference was detected between mild fibrosis and advanced stages of steatohepatitis (95 ± 9 μg/dL *vs* 72 ± 28 μg/dL); however, this marker was unable to differentiate between patients and non-patients[153]. These results confirmed the results of another study conducted 4 years earlier which had shown that the mean DHEA-S level in patients with degree 2-4 of steatohepatitis was significantly lower than in those suffering from milder stages (0-1) and that the mean of DHEA-S level decreased stage by stage with increase in the fibrosis stages. In this study, a DHEA-S value of more than 1.0 μg/mL showed sensitivity of 95% and specificity of 58% for diagnosis of advanced stages of NAFLD[149]. In another study, sensitivity, specificity, PPV and NPV were 76.5%, 73.3%, 29.5% and 95.5%, respectively, considering a cutoff point of equal or less than 66 mg/dL[65]. In the same direction, Sumida *et al*[1] in another article suggested that patients with DHEA-S serum level of more than 66 μg/dL are unlikely to suffer from advanced stages of NAFLD.

It can be generally concluded that increased level of serum DHEA-S may be a part of the pathophysiology of NAFLD and could play a role in the development of this disease[23]. However, considering metabolic and intracellular effects reported for this hormone, such as its protective effect against oxidative damage in animal models which is applied by reducing concentration of MDA and increasing activity of superoxide dismutase in hepatocytes and total concentration of glutathione, it may have an important role in prevention of histological progress of NAFLD; its blood level variations may also be well correlated with different histological patterns of patients with similar metabolic profile, age and sex[149]. Considering these findings, it is not surprising if serum level of DHEA developed into a successful predictor for diagnosis and staging of hepatic fibrosis in NAFLD patients in the future.

### *Fibrinogen-like protein 2*

Fibrinogen-like protein 2 (FGL2) is a new member of the fibrinogen-like protein family. A study investigating this protein in patients with NAFLD showed that plasma level of this protein was considerably higher in patients with steatohepatitis, but there was no difference between patients with simple steatosis and the control group. Results of this study indicated a potential role for FGL2 level in the diagnosis of severe forms of fatty liver diseases and differentiated between simple steatosis and steatohepatitis[154].

## CONCLUSION

Although some of the reported markers such as adiponectin, hyaluronic acid, CK-18 and DHEA have shown promising results, at present, it does not seem that any of these individual markers are reliable enough to be considered as a marker for diagnosis and staging of NAFLD. An ideal biomarker should have specific characteristics such as simplicity, accessibility, accuracy, repeatability and cost-effectiveness, some of which are not applicable to any of these markers[72,155]. Also, a reliable marker should be able to provide physicians with clear information, have high diagnostic power for differentiation between different stages of the disease, and should have been validated in large prospective trials[156,157]. It should be noted, as well, that selection of a suitable test depends on the clinical demand of the healthcare provider.

If ruling out of the diagnosis of NAFLD and/or its staging is the goal, applying the markers with higher sensitivity and NPV is recommended; however, if the main aim is diagnosis of the disease in suspected patients, the test's specificity and PPV would be the most important factors that should be taken into consideration. Furthermore, determining a suitable cutoff point for a test is usually based on a tradeoff between sensitivity (true positive) and specificity (true negative) results of the test. In fact, the optimal cutoff point has the highest sensitivity and specificity, but this point can hardly be obtained for a marker. Therefore, based on different cutoff values, the diagnostic power of a test and its productivity will be different. Paying attention to different cutoff values suggested for each marker while comparing them is important.

Considering the weaknesses of every individual marker for diagnosis and staging of the disease, it seems that the present trend would be toward combining some of these serum markers together, and even adding some other parameters, such as demographic variables or radiologic results, and developing a mathematical model which has higher diagnostic power and accuracy[72,158].

## REFERENCES

1 **Sumida Y**, Yoshikawa T, Tanaka S, Taketani H, Kanemasa K, Nishimura T, Yamaguchi K, Mitsuyoshi H, Yasui K, Minami M, Naito Y, Itoh Y. The 'donations for decreased ALT (D4D)' prosocial behavior incentive scheme for NAFLD patients. *J Public Health* (Oxf) 2014; **36**: 629-634 [PMID: 24099735 DOI: 10.1093/pubmed/fdt098]

2 **Moore JB**. Non-alcoholic fatty liver disease: the hepatic consequence

of obesity and the metabolic syndrome. *Proc Nutr Soc* 2010; **69**: 211-220 [PMID: 20158939 DOI: 10.1017/S0029665110000030]
3 **Paschos P**, Paletas K. Non alcoholic fatty liver disease and metabolic syndrome. *Hippokratia* 2009; **13**: 9-19 [PMID: 19240815]
4 **Pulzi FB**, Cisternas R, Melo MR, Ribeiro CM, Malheiros CA, Salles JE. New clinical score to diagnose nonalcoholic steatohepatitis in obese patients. *Diabetol Metab Syndr* 2011; **3**: 3 [PMID: 21345221 DOI: 10.1186/1758-5996-3-3]
5 **Collier J**. Non-alcoholic fatty liver disease. *Medicine* 2007; **35**: 86-88 [DOI: 10.1016/j.mpmed.2006.11.010]
6 **Tevar AD**, Clarke C, Wang J, Rudich SM, Woodle ES, Lentsch AB, Edwards ML. Clinical review of nonalcoholic steatohepatitis in liver surgery and transplantation. *J Am Coll Surg* 2010; **210**: 515-526 [PMID: 20347746 DOI: 10.1016/j.jamcollsurg.2010.01.020]
7 **Chitturi S**, Farrell GC, Hashimoto E, Saibara T, Lau GK, Sollano JD. Non-alcoholic fatty liver disease in the Asia-Pacific region: definitions and overview of proposed guidelines. *J Gastroenterol Hepatol* 2007; **22**: 778-787 [PMID: 17565630 DOI: 10.1111/j.1440-1746.2007.05001.x]
8 **Fan JG**, Farrell GC. Epidemiology of non-alcoholic fatty liver disease in China. *J Hepatol* 2009; **50**: 204-210 [PMID: 19014878 DOI: 10.1016/j.jhep.2008.10.010]
9 **Westerbacka J**, Cornér A, Tiikkainen M, Tamminen M, Vehkavaara S, Häkkinen AM, Fredriksson J, Yki-Järvinen H. Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. *Diabetologia* 2004; **47**: 1360-1369 [PMID: 15309287 DOI: 10.1007/s00125-004-1460-1]
10 **De Hewavisenthi SJ**, Dassanayaka AS, De Silva HJ. Clinical, biochemical and histological characteristics of a Sri Lankan population of non-alcoholic steatohepatitis (NASH) patients. *Ceylon Med J* 2005; **50**: 113-116 [PMID: 16252575]
11 **Baranova A**, Younossi ZM. The future is around the corner: Noninvasive diagnosis of progressive nonalcoholic steatohepatitis. *Hepatology* 2008; **47**: 373-375 [PMID: 18220279 DOI: 10.1002/hep.22140]
12 **Poynard T**, Ratziu V, Naveau S, Thabut D, Charlotte F, Messous D, Capron D, Abella A, Massard J, Ngo Y, Munteanu M, Mercadier A, Manns M, Albrecht J. The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis. *Comp Hepatol* 2005; **4**: 10 [PMID: 16375767 DOI: 10.1186/1476-5926-4-10]
13 **Castera L**. Non-invasive diagnosis of steatosis and fibrosis. *Diabetes Metab* 2008; **34**: 674-679 [PMID: 19195629 DOI: 10.1016/S1262-3636(08)74603-2]
14 **Bondini S**, Kleiner DE, Goodman ZD, Gramlich T, Younossi ZM. Pathologic assessment of non-alcoholic fatty liver disease. *Clin Liver Dis* 2007; **11**: 17-23, vii [PMID: 17544969 DOI: 10.1016/j.cld.2007.02.002]
15 **Ratziu V**, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F, Poynard T. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005; **128**: 1898-1906 [PMID: 15940625]
16 **Manousou P**, Kalambokis G, Grillo F, Watkins J, Xirouchakis E, Pleguezuelo M, Leandro G, Arvaniti V, Germani G, Patch D, Calvaruso V, Mikhailidis DP, Dhillon AP, Burroughs AK. Serum ferritin is a discriminant marker for both fibrosis and inflammation in histologically proven non-alcoholic fatty liver disease patients. *Liver Int* 2011; **31**: 730-739 [PMID: 21457446 DOI: 10.1111/j.1478-3231.2011.02488.x]
17 **Wilson S**, Chalasani N. Noninvasive markers of advanced histology in nonalcoholic fatty liver disease: are we there yet? *Gastroenterology* 2007; **133**: 1377-1378; discussion 1378-1379 [PMID: 17919509 DOI: 10.1053/j.gastro.2007.08.045]
18 **Díez-Vallejo J**, Comas-Fuentes A. Asymptomatic hypertransaminasemia in patients in primary care. *Rev Esp Enferm Dig* 2011; **103**: 530-535 [PMID: 22054268]
19 **Hossain N**, Afendy A, Stepanova M, Nader F, Srishord M, Rafiq N, Goodman Z, Younossi Z. Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 1224-1229, 1229e1-e2 [PMID: 19559819 DOI: 10.1016/j.cgh.2009.06.007]
20 **Wilfred de Alwis NM**, Day CP. Genetics of alcoholic liver disease and nonalcoholic fatty liver disease. *Semin Liver Dis* 2007; **27**: 44-54 [PMID: 17295176 DOI: 10.1055/s-2006-960170]
21 **Ioannou GN**, Boyko EJ, Lee SP. The prevalence and predictors of elevated serum aminotransferase activity in the United States in 1999-2002. *Am J Gastroenterol* 2006; **101**: 76-82 [PMID: 16405537 DOI: 10.1111/j.1572-0241.2005.00341.x]
22 **Esteghamati A**, Jamali A, Khalilzadeh O, Noshad S, Khalili M, Zandieh A, Morteza A, Nakhjavani M. Metabolic syndrome is linked to a mild elevation in liver aminotransferases in diabetic patients with undetectable non-alcoholic fatty liver disease by ultrasound. *Diabetol Metab Syndr* 2010; **2**: 65 [PMID: 21047423 DOI: 10.1186/1758-5996-2-65]
23 **Koga M**, Saito H, Mukai M, Saibara T, Kasayama S. Serum dehydroepiandrosterone sulphate levels in patients with non-alcoholic fatty liver disease. *Intern Med* 2011; **50**: 1657-1661 [PMID: 21841322]
24 **Pourshams A**, Malekzadeh R, Monavvari A, Akbari MR, Mohamadkhani A, Yarahmadi S, Seddighi N, Mohamadnejad M, Sotoudeh M, Madjlessi A. Prevalence and etiology of persistently elevated alanine aminotransferase levels in healthy Iranian blood donors. *J Gastroenterol Hepatol* 2005; **20**: 229-233 [PMID: 15683425 DOI: 10.1111/j.1440-1746.2004.03511.x]
25 **Fracanzani AL**, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, Bertelli C, Fatta E, Bignamini D, Marchesini G, Fargion S. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. *Hepatology* 2008; **48**: 792-798 [PMID: 18752331 DOI: 10.1002/hep.22429]
26 **Neuschwander-Tetri BA**, Clark JM, Bass NM, Van Natta ML, Unalp-Arida A, Tonascia J, Zein CO, Brunt EM, Kleiner DE, McCullough AJ, Sanyal AJ, Diehl AM, Lavine JE, Chalasani N, Kowdley KV. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. *Hepatology* 2010; **52**: 913-924 [PMID: 20648476 DOI: 10.1002/hep.23784]
27 **Ratziu V**, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T. Liver fibrosis in overweight patients. *Gastroenterology* 2000; **118**: 1117-1123 [PMID: 10833486]
28 **Sumida Y**, Yoneda M, Hyogo H, Itoh Y, Ono M, Fujii H, Eguchi Y, Suzuki Y, Aoki N, Kanemasa K, Fujita K, Chayama K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Yoshikawa T, Okanoue T. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol* 2012; **12**: 2 [PMID: 22221544 DOI: 10.1186/1471-230X-12-2]
29 **Ratziu V**, Giral P, Munteanu M, Messous D, Mercadier A, Bernard M, Morra R, Imbert-Bismut F, Bruckert E, Poynard T. Screening for liver disease using non-invasive biomarkers (FibroTest, SteatoTest and NashTest) in patients with hyperlipidaemia. *Aliment Pharmacol Ther* 2007; **25**: 207-218 [PMID: 17229244 DOI: 10.1111/j.1365-2036.2006.03182.x]
30 **Calès P**, Boursier J, Chaigneau J, Lainé F, Sandrini J, Michalak S, Hubert I, Dib N, Oberti F, Bertrais S, Hunault G, Cavaro-Ménard C, Gallois Y, Deugnier Y, Rousselet MC. Diagnosis of different liver fibrosis characteristics by blood tests in non-alcoholic fatty liver disease. *Liver Int* 2010; **30**: 1346-1354 [PMID: 20666992 DOI: 10.1111/j.1478-3231.2010.02314.x]
31 **Browning JD**. Statins and hepatic steatosis: perspectives from the Dallas Heart Study. *Hepatology* 2006; **44**: 466-471 [PMID: 16871575 DOI: 10.1002/hep.21248]
32 **Bedogni G**, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* 2005; **42**: 44-52 [PMID: 15895401 DOI: 10.1002/hep.20734]
33 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [PMID: 15565570 DOI: 10.1002/hep.20466]

34 **Gholam PM**, Flancbaum L, Machan JT, Charney DA, Kotler DP. Nonalcoholic fatty liver disease in severely obese subjects. *Am J Gastroenterol* 2007; **102**: 399-408 [PMID: 17311652 DOI: 10.1111/j.1572-0241.2006.01041.x]

35 **Chang Y**, Ryu S, Sung E, Jang Y. Higher concentrations of alanine aminotransferase within the reference interval predict nonalcoholic fatty liver disease. *Clin Chem* 2007; **53**: 686-692 [PMID: 17272484 DOI: 10.1373/clinchem.2006.081257]

36 **Leite NC**, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int* 2009; **29**: 113-119 [PMID: 18384521 DOI: 10.1111/j.1478-3231.2008.01718.x]

37 **M'Kada H**, Munteanu M, Perazzo H, Ngo Y, Ramanujam N, Imbert-Bismut F, Ratziu V, Bonnefont-Rousselot D, Souberbielle B, Schuppe-Koistinen I, Poynard T. What are the best reference values for a normal serum alanine transaminase activity (ALT)? Impact on the presumed prevalence of drug induced liver injury (DILI). *Regul Toxicol Pharmacol* 2011; **60**: 290-295 [PMID: 21539883 DOI: 10.1016/j.yrtph.2011.04.002]

38 **Prati D**, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, Vianello L, Zanuso F, Mozzi F, Milani S, Conte D, Colombo M, Sirchia G. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med* 2002; **137**: 1-10 [PMID: 12093239]

39 **Miyake T**, Kumagi T, Hirooka M, Koizumi M, Furukawa S, Ueda T, Tokumoto Y, Ikeda Y, Abe M, Kitai K, Hiasa Y, Matsuura B, Onji M. Metabolic markers and ALT cutoff level for diagnosing nonalcoholic fatty liver disease: a community-based cross-sectional study. *J Gastroenterol* 2012; **47**: 696-703 [PMID: 22331365 DOI: 10.1007/s00535-012-0534-y]

40 **Kaplan MM**. Alanine aminotransferase levels: what's normal? *Ann Intern Med* 2002; **137**: 49-51 [PMID: 12093245 DOI: 10.7326/0003-4819-137-1-200207020-00012]

41 **Uslusoy HS**, Nak SG, Gülten M, Biyikli Z. Non-alcoholic steatohepatitis with normal aminotransferase values. *World J Gastroenterol* 2009; **15**: 1863-1868 [PMID: 19370784 DOI: 10.3748/wjg.15.1863]

42 **Kayadibi H**, Gültepe M, Yasar B, Ince AT, Ozcan O, Ipcioglu OM, Kurdas OO, Bolat B, Benek YZ, Guveli H, Atalay S, Ozkara S, Keskin O. Diagnostic value of serum prolidase enzyme activity to predict the liver histological lesions in non-alcoholic fatty liver disease: a surrogate marker to distinguish steatohepatitis from simple steatosis. *Dig Dis Sci* 2009; **54**: 1764-1771 [PMID: 18989777 DOI: 10.1007/s10620-008-0535-0]

43 **Riquelme A**, Arrese M, Soza A, Morales A, Baudrand R, Pérez-Ayuso RM, González R, Alvarez M, Hernández V, García-Zattera MJ, Otarola F, Medina B, Rigotti A, Miquel JF, Marshall G, Nervi F. Non-alcoholic fatty liver disease and its association with obesity, insulin resistance and increased serum levels of C-reactive protein in Hispanics. *Liver Int* 2009; **29**: 82-88 [PMID: 18647235 DOI: 10.1111/j.1478-3231.2008.01823.x]

44 **Shi JP**, Xun YH, Hu CB, Zhang L, Liu H, Lou GQ, Fan JG. Clinical and histological features of non-alcoholic fatty liver disease. *Zhonghua Ganzangbing Zazhi* 2009; **17**: 812-816 [PMID: 19958638]

45 **Prashanth M**, Ganesh HK, Vima MV, John M, Bandgar T, Joshi SR, Shah SR, Rathi PM, Joshi AS, Thakkar H, Menon PS, Shah NS. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. *J Assoc Physicians India* 2009; **57**: 205-210 [PMID: 19588648]

46 **Kashyap SR**, Diab DL, Baker AR, Yerian L, Bajaj H, Gray-McGuire C, Schauer PR, Gupta M, Feldstein AE, Hazen SL, Stein CM. Triglyceride levels and not adipokine concentrations are closely related to severity of nonalcoholic fatty liver disease in an obesity surgery cohort. *Obesity* (Silver Spring) 2009; **17**: 1696-1701 [PMID: 19360015 DOI: 10.1038/oby.2009.89]

47 **Charatcharoenwitthaya P**, Lindor KD, Angulo P. The spontaneous course of liver enzymes and its correlation in nonalcoholic fatty liver disease. *Dig Dis Sci* 2012; **57**: 1925-1931 [PMID: 22373863 DOI: 10.1007/s10620-012-2098-3]

48 **Kaneda H**, Hashimoto E, Yatsuji S, Tokushige K, Shiratori K. Hyaluronic acid levels can predict severe fibrosis and platelet counts can predict cirrhosis in patients with nonalcoholic fatty liver disease. *J Gastroenterol Hepatol* 2006; **21**: 1459-1465 [PMID: 16911693 DOI: 10.1111/j.1440-1746.2006.04447.x]

49 **Hsieh MH**, Ho CK, Hou NJ, Hsieh MY, Lin WY, Yang JF, Chiu CC, Huang JF, Chang NC, Wang CL, Dai CY, Chuang WL, Yu ML. Abnormal liver function test results are related to metabolic syndrome and BMI in Taiwanese adults without chronic hepatitis B or C. *Int J Obes* (Lond) 2009; **33**: 1309-1317 [PMID: 19752878 DOI: 10.1038/ijo.2009.172]

50 **Fan JG**, Saibara T, Chitturi S, Kim BI, Sung JJ, Chutaputti A. What are the risk factors and settings for non-alcoholic fatty liver disease in Asia-Pacific? *J Gastroenterol Hepatol* 2007; **22**: 794-800 [PMID: 17498218 DOI: 10.1111/j.1440-1746.2007.04952.x]

51 **Munteanu M**, Ratziu V, Morra R, Messous D, Imbert-Bismut F, Poynard T. Noninvasive biomarkers for the screening of fibrosis, steatosis and steatohepatitis in patients with metabolic risk factors: FibroTest-FibroMax experience. *J Gastrointestin Liver Dis* 2008; **17**: 187-191 [PMID: 18568141]

52 **Poynard T**, Ratziu V, Charlotte F, Messous D, Munteanu M, Imbert-Bismut F, Massard J, Bonyhay L, Tahiri M, Thabut D, Cadranel JF, Le Bail B, de Ledinghen V. Diagnostic value of biochemical markers (NashTest) for the prediction of non alcoholo steato hepatitis in patients with non-alcoholic fatty liver disease. *BMC Gastroenterol* 2006; **6**: 34 [PMID: 17096854 DOI: 10.1186/1471-230X-6-34]

53 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854 [PMID: 17393509 DOI: 10.1002/hep.21496]

54 **Wong VW**, Wong GL, Chim AM, Tse AM, Tsang SW, Hui AY, Choi PC, Chan AW, So WY, Chan FK, Sung JJ, Chan HL. Validation of the NAFLD fibrosis score in a Chinese population with low prevalence of advanced fibrosis. *Am J Gastroenterol* 2008; **103**: 1682-1688 [PMID: 18616651 DOI: 10.1111/j.1572-0241.2008.01933.x]

55 **Calès P**, Lainé F, Boursier J, Deugnier Y, Moal V, Oberti F, Hunault G, Rousselet MC, Hubert I, Laafi J, Ducluzeaux PH, Lunel F. Comparison of blood tests for liver fibrosis specific or not to NAFLD. *J Hepatol* 2009; **50**: 165-173 [PMID: 18977552 DOI: 10.1016/j.jhep.2008.07.035]

56 **Younossi ZM**, Page S, Rafiq N, Birerdinc A, Stepanova M, Hossain N, Afendy A, Younoszai Z, Goodman Z, Baranova A. A biomarker panel for non-alcoholic steatohepatitis (NASH) and NASH-related fibrosis. *Obes Surg* 2011; **21**: 431-439 [PMID: 20532833 DOI: 10.1007/s11695-010-0204-1]

57 **Palekar NA**, Naus R, Larson SP, Ward J, Harrison SA. Clinical model for distinguishing nonalcoholic steatohepatitis from simple steatosis in patients with nonalcoholic fatty liver disease. *Liver Int* 2006; **26**: 151-156 [PMID: 16448452 DOI: 10.1111/j.1478-3231.2005.01209.x]

58 **Harrison SA**, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008; **57**: 1441-1447 [PMID: 18390575 DOI: 10.1136/gut.2007.146019]

59 **Kruger FC**, Daniels CR, Kidd M, Swart G, Brundyn K, van Rensburg C, Kotze M. APRI: a simple bedside marker for advanced fibrosis that can avoid liver biopsy in patients with NAFLD/NASH. *S Afr Med J* 2011; **101**: 477-480 [PMID: 21920102]

60 **Yoshida M**. Novel role of NPC1L1 in the regulation of hepatic metabolism: potential contribution of ezetimibe in NAFLD/NASH treatment. *Curr Vasc Pharmacol* 2011; **9**: 121-123 [PMID: 21044016]

61 **Rafiq N**, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, Younossi ZM. Long-term follow-up of patients with nonalcoholic fatty liver. *Clin Gastroenterol Hepatol* 2009; **7**: 234-238 [PMID: 19049831 DOI: 10.1016/j.cgh.2008.11.005]

62 **Fierbinteanu-Braticevici C**, Baicus C, Tribus L, Papacocea

R. Predictive factors for nonalcoholic steatohepatitis (NASH) in patients with nonalcoholic fatty liver disease (NAFLD). *J Gastrointestin Liver Dis* 2011; **20**: 153-159 [PMID: 21725512]
63 **Younossi ZM**, Gramlich T, Matteoni CA, Boparai N, McCullough AJ. Nonalcoholic fatty liver disease in patients with type 2 diabetes. *Clin Gastroenterol Hepatol* 2004; **2**: 262-265 [PMID: 15017611]
64 **Suzuki A**, Angulo P, Lymp J, Li D, Satomura S, Lindor K. Hyaluronic acid, an accurate serum marker for severe hepatic fibrosis in patients with non-alcoholic fatty liver disease. *Liver Int* 2005; **25**: 779-786 [PMID: 15998429 DOI: 10.1111/j.1478-3231.2005.01064.x]
65 **Sumida Y**, Yonei Y, Kanemasa K, Hara T, Inada Y, Sakai K, Imai S, Hibino S, Yamaguchi K, Mitsuyoshi H, Yasui K, Minami M, Itoh Y, Naito Y, Yoshikawa T, Okanoue T. Lower circulating levels of dehydroepiandrosterone, independent of insulin resistance, is an important determinant of severity of non-alcoholic steatohepatitis in Japanese patients. *Hepatol Res* 2010; **40**: 901-910 [PMID: 20887595 DOI: 10.1111/j.1872-034X.2010.00704.x]
66 **Mawatari H**, Yoneda M, Kirikoshi H, Maeda S, Nakajima A, Saito S. Thrombocytopenia is more severe in patients with chronic hepatitis C than in patients with nonalcoholic fatty liver disease. *J Gastroenterol* 2012; **47**: 606-607; author reply 608 [PMID: 22388885]
67 **Yoneda M**, Fujii H, Sumida Y, Hyogo H, Itoh Y, Ono M, Eguchi Y, Suzuki Y, Aoki N, Kanemasa K, Imajo K, Chayama K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Yoshikawa T, Okanoue T. Platelet count for predicting fibrosis in nonalcoholic fatty liver disease. *J Gastroenterol* 2011; **46**: 1300-1306 [PMID: 21750883 DOI: 10.1007/s00535-011-0436-4]
68 **Celikbilek M**, Gürsoy S, Deniz K, Karaman A, Zararsiz G, Yurci A. Mean platelet volume in biopsy-proven non-alcoholic fatty liver disease. *Platelets* 2013; **24**: 194-199 [PMID: 22646469 DOI: 10.3109/09537104.2012.688898]
69 **Ozhan H**, Aydin M, Yazici M, Yazgan O, Basar C, Gungor A, Onder E. Mean platelet volume in patients with non-alcoholic fatty liver disease. *Platelets* 2010; **21**: 29-32 [PMID: 19947902 DOI: 10.3109/09537100903391023]
70 **Shin WY**, Jung DH, Shim JY, Lee HR. The association between non-alcoholic hepatic steatosis and mean platelet volume in an obese Korean population. *Platelets* 2011; **22**: 442-446 [PMID: 21751850 DOI: 10.3109/09537104.2010.540049]
71 **Kilciler G**, Genc H, Tapan S, Ors F, Kara M, Karadurmus N, Ercin CN, Karslioglu Y, Kilic S, Bagci S, Erbil MK, Dogru T. Mean platelet volume and its relationship with carotid atherosclerosis in subjects with non-alcoholic fatty liver disease. *Ups J Med Sci* 2010; **115**: 253-259 [PMID: 20731535 DOI: 10.3109/03009734.2010.500062]
72 **Hashimoto E**, Farrell GC. Will non-invasive markers replace liver biopsy for diagnosing and staging fibrosis in non-alcoholic steatohepatitis? *J Gastroenterol Hepatol* 2009; **24**: 501-503 [PMID: 19368628 DOI: 10.1111/j.1440-1746.2009.05806.x]
73 **Siebler J**, Galle PR, Weber MM. The gut-liver-axis: endotoxemia, inflammation, insulin resistance and NASH. *J Hepatol* 2008; **48**: 1032-1034 [PMID: 18468548 DOI: 10.1016/j.jhep.2008.03.007]
74 **Kogiso T**, Moriyoshi Y, Shimizu S, Nagahara H, Shiratori K. High-sensitivity C-reactive protein as a serum predictor of nonalcoholic fatty liver disease based on the Akaike Information Criterion scoring system in the general Japanese population. *J Gastroenterol* 2009; **44**: 313-321 [PMID: 19271113 DOI: 10.1007/s00535-009-0002-5]
75 **Kerner A**, Avizohar O, Sella R, Bartha P, Zinder O, Markiewicz W, Levy Y, Brook GJ, Aronson D. Association between elevated liver enzymes and C-reactive protein: possible hepatic contribution to systemic inflammation in the metabolic syndrome. *Arterioscler Thromb Vasc Biol* 2005; **25**: 193-197 [PMID: 15499043 DOI: 10.1161/01.ATV.0000148324.63685.6a]
76 **Yoneda M**, Mawatari H, Fujita K, Iida H, Yonemitsu K, Kato S, Takahashi H, Kirikoshi H, Inamori M, Nozaki Y, Abe Y, Kubota K, Saito S, Iwasaki T, Terauchi Y, Togo S, Maeyama S, Nakajima A. High-sensitivity C-reactive protein is an independent clinical feature of nonalcoholic steatohepatitis (NASH) and also of the severity of fibrosis in NASH. *J Gastroenterol* 2007; **42**: 573-582 [PMID: 17653654 DOI: 10.1007/s00535-007-2060-x]
77 **Uchihara M**, Izumi N. [High-sensitivity C-reactive protein (hs-CRP): a promising biomarker for the screening of non-alcoholic steatohepatitis (NASH)]. *Nihon Rinsho* 2006; **64**: 1133-1138 [PMID: 16768121]
78 **Zimmermann E**, Anty R, Tordjman J, Verrijken A, Gual P, Tran A, Iannelli A, Gugenheim J, Bedossa P, Francque S, Le Marchand-Brustel Y, Clement K, Van Gaal L, Sørensen TI, Jess T. C-reactive protein levels in relation to various features of non-alcoholic fatty liver disease among obese patients. *J Hepatol* 2011; **55**: 660-665 [PMID: 21238518 DOI: 10.1016/j.jhep.2010.12.017]
79 **Park SH**, Kim BI, Yun JW, Kim JW, Park DI, Cho YK, Sung IK, Park CY, Sohn CI, Jeon WK, Kim H, Rhee EJ, Lee WY, Kim SW. Insulin resistance and C-reactive protein as independent risk factors for non-alcoholic fatty liver disease in non-obese Asian men. *J Gastroenterol Hepatol* 2004; **19**: 694-698 [PMID: 15151626 DOI: 10.1111/j.1440-1746.2004.03362.x]
80 **Anty R**, Bekri S, Luciani N, Saint-Paul MC, Dahman M, Iannelli A, Amor IB, Staccini-Myx A, Huet PM, Gugenheim J, Sadoul JL, Le Marchand-Brustel Y, Tran A, Gual P. The inflammatory C-reactive protein is increased in both liver and adipose tissue in severely obese patients independently from metabolic syndrome, Type 2 diabetes, and NASH. *Am J Gastroenterol* 2006; **101**: 1824-1833 [PMID: 16790033 DOI: 10.1111/j.1572-0241.2006.00724.x]
81 **Park HS**, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. *Diabetes Res Clin Pract* 2005; **69**: 29-35 [PMID: 15955385 DOI: 10.1016/j.diabres.2004.11.007]
82 **Sivapackianathan R**, Asivatham AJ, Al-Mahtab M, Chowdhury TA. Association between non-alcoholic fatty liver disease and metabolic syndrome. *Int J Hepatol* 2010; **1**: 17-24
83 **Khera A**, Vega GL, Das SR, Ayers C, McGuire DK, Grundy SM, de Lemos JA. Sex differences in the relationship between C-reactive protein and body fat. *J Clin Endocrinol Metab* 2009; **94**: 3251-3258 [PMID: 19567538 DOI: 10.1210/jc.2008-2406]
84 **Lakoski SG**, Cushman M, Criqui M, Rundek T, Blumenthal RS, D'Agostino RB, Herrington DM. Gender and C-reactive protein: data from the Multiethnic Study of Atherosclerosis (MESA) cohort. *Am Heart J* 2006; **152**: 593-598 [PMID: 16923436 DOI: 10.1016/j.ahj.2006.02.015]
85 **Khera A**, McGuire DK, Murphy SA, Stanek HG, Das SR, Vongpatanasin W, Wians FH, Grundy SM, de Lemos JA. Race and gender differences in C-reactive protein levels. *J Am Coll Cardiol* 2005; **46**: 464-469 [PMID: 16053959 DOI: 10.1016/j.jacc.2005.04.051]
86 **Das I**. Raised C-reactive protein levels in serum from smokers. *Clin Chim Acta* 1985; **153**: 9-13 [PMID: 2416495]
87 **Tsochatzis EA**, Papatheodoridis GV, Archimandritis AJ. Adipokines in nonalcoholic steatohepatitis: from pathogenesis to implications in diagnosis and therapy. *Mediators Inflamm* 2009; **2009**: 831670 [PMID: 19753129 DOI: 10.1155/2009/831670]
88 **Oda E**, Watanabe K. High-sensitivity C-reactive protein and metabolic syndrome (insulin resistance syndrome), including nonalcoholic steatohepatitis. *J Gastroenterol* 2008; **43**: 312-313; author reply 313 [PMID: 18458848 DOI: 10.1007/s00535-008-2175-8]
89 **Kowdley KV**, Belt P, Wilson LA, Yeh MM, Neuschwander-Tetri BA, Chalasani N, Sanyal AJ, Nelson JE. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2012; **55**: 77-85 [PMID: 21953442 DOI: 10.1002/hep.24706]
90 **Oh MK**, Winn J, Poordad F. Review article: diagnosis and treatment of non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2008; **28**: 503-522 [PMID: 18532991 DOI: 10.1111/j.1365-2036.2008.03752.x]
91 **Sumida Y**, Yoneda M, Hyogo H, Yamaguchi K, Ono M, Fujii H, Eguchi Y, Suzuki Y, Imai S, Kanemasa K, Fujita K, Chayama K, Yasui K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Okanoue T. A simple clinical scoring system using ferritin, fasting insulin, and type IV collagen 7S for predicting steatohepatitis in nonalcoholic

fatty liver disease. *J Gastroenterol* 2011; **46**: 257-268 [PMID: 20842510 DOI: 10.1007/s00535-010-0305-6]
92 **Polyzos SA**, Kountouras J, Patsiaoura K, Katsiki E, Zafeiriadou E, Deretzi G, Zavos C, Gavalas E, Katsinelos P, Mane V, Slavakis A. Serum homocysteine levels in patients with nonalcoholic fatty liver disease. *Ann Hepatol* 2012; **11**: 68-76 [PMID: 22166563]
93 **Blendis L**, Oren R, Halpern Z. NASH: Can we iron out the pathogenesis? *Gastroenterology* 2000; **118**: 981-983 [PMID: 10784602 DOI: 10.1016/S0016-5085(00)70189-2]
94 **Yoneda M**, Mawatari H, Fujita K, Yonemitsu K, Kato S, Takahashi H, Kirikoshi H, Inamori M, Nozaki Y, Abe Y, Kubota K, Saito S, Iwasaki T, Terauchi Y, Togo S, Maeyama S, Nakajima A. Type IV collagen 7s domain is an independent clinical marker of the severity of fibrosis in patients with nonalcoholic steatohepatitis before the cirrhotic stage. *J Gastroenterol* 2007; **42**: 375-381 [PMID: 17530362 DOI: 10.1007/s00535-007-2014-3]
95 **Yoneda M**, Uchiyama T, Kato S, Endo H, Fujita K, Yoneda K, Mawatari H, Iida H, Takahashi H, Kirikoshi H, Inamori M, Nozaki Y, Kobayashi N, Kubota K, Saito S, Maeyama S, Sagara M, Aburatani H, Kodama T, Nakajima A. Plasma Pentraxin3 is a novel marker for nonalcoholic steatohepatitis (NASH). *BMC Gastroenterol* 2008; **8**: 53 [PMID: 19014569 DOI: 10.1186/1471-230X-8-53]
96 **Polyzos SA**, Kountouras J, Zavos C. Nonalcoholic fatty liver disease: the pathogenetic roles of insulin resistance and adipocytokines. *Curr Mol Med* 2009; **9**: 299-314 [PMID: 19355912]
97 **Yalniz M**, Bahcecioglu IH, Ataseven H, Ustundag B, Ilhan F, Poyrazoglu OK, Erensoy A. Serum adipokine and ghrelin levels in nonalcoholic steatohepatitis. *Mediators Inflamm* 2006; **2006**: 34295 [PMID: 17392582 DOI: 10.1155/MI/2006/34295]
98 **Jarrar MH**, Baranova A, Collantes R, Ranard B, Stepanova M, Bennett C, Fang Y, Elariny H, Goodman Z, Chandhoke V, Younossi ZM. Adipokines and cytokines in non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2008; **27**: 412-421 [PMID: 18081738 DOI: 10.1111/j.1365-2036.2007.03586.x]
99 **Tilg H**. The role of cytokines in non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 179-185 [PMID: 20460908 DOI: 10.1159/000282083]
100 **Hui JM**, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology* 2004; **40**: 46-54 [PMID: 15239085 DOI: 10.1002/hep.20280]
101 **Buechler C**, Wanninger J, Neumeier M. Adiponectin, a key adipokine in obesity related liver diseases. *World J Gastroenterol* 2011; **17**: 2801-2811 [PMID: 21734787 DOI: 10.3748/wjg.v17.i23.2801]
102 **Aller R**, de Luis DA, Fernandez L, Calle F, Velayos B, Olcoz JL, Izaola O, Sagrado MG, Conde R, Gonzalez JM. Influence of insulin resistance and adipokines in the grade of steatosis of nonalcoholic fatty liver disease. *Dig Dis Sci* 2008; **53**: 1088-1092 [PMID: 17934820 DOI: 10.1007/s10620-007-9981-3]
103 **Musso G**, Gambino R, Durazzo M, Biroli G, Carello M, Fagà E, Pacini G, De Michieli F, Rabbione L, Premoli A, Cassader M, Pagano G. Adipokines in NASH: postprandial lipid metabolism as a link between adiponectin and liver disease. *Hepatology* 2005; **42**: 1175-1183 [PMID: 16231364 DOI: 10.1002/hep.20896]
104 **Shimada M**, Kawahara H, Ozaki K, Fukura M, Yano H, Tsuchishima M, Tsutsumi M, Takase S. Usefulness of a combined evaluation of the serum adiponectin level, HOMA-IR, and serum type IV collagen 7S level to predict the early stage of nonalcoholic steatohepatitis. *Am J Gastroenterol* 2007; **102**: 1931-1938 [PMID: 17511754 DOI: 10.1111/j.1572-0241.2007.01322.x]
105 **Tokushige K**, Hashimoto E, Noto H, Yatsuji S, Taniai M, Torii N, Shiratori K. Influence of adiponectin gene polymorphisms in Japanese patients with non-alcoholic fatty liver disease. *J Gastroenterol* 2009; **44**: 976-982 [PMID: 19484180 DOI: 10.1007/s00535-009-0085-z]
106 **Kaser S**, Moschen A, Cayon A, Kaser A, Crespo J, Pons-Romero F, Ebenbichler CF, Patsch JR, Tilg H. Adiponectin and its receptors in non-alcoholic steatohepatitis. *Gut* 2005; **54**: 117-121 [PMID: 15591515 DOI: 10.1136/gut.2003.037010]
107 **Younossi ZM**, Jarrar M, Nugent C, Randhawa M, Afendy M, Stepanova M, Rafiq N, Goodman Z, Chandhoke V, Baranova A. A novel diagnostic biomarker panel for obesity-related nonalcoholic steatohepatitis (NASH). *Obes Surg* 2008; **18**: 1430-1437 [PMID: 18500507 DOI: 10.1007/s11695-008-9506-y]
108 **Bethanis SK**, Theocharis SE. Leptin in the field of hepatic fibrosis: a pivotal or an incidental player? *Dig Dis Sci* 2006; **51**: 1685-1696 [PMID: 16958000 DOI: 10.1007/s10620-006-9126-0]
109 **Uygun A**, Kadayifci A, Yesilova Z, Erdil A, Yaman H, Saka M, Deveci MS, Bagci S, Gulsen M, Karaeren N, Dagalp K. Serum leptin levels in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2000; **95**: 3584-3589 [PMID: 11151896 DOI: 10.1111/j.1572-0241.2000.03297.x]
110 **Polyzos SA**, Kountouras J, Zavos C, Deretzi G. The potential adverse role of leptin resistance in nonalcoholic fatty liver disease: a hypothesis based on critical review of the literature. *J Clin Gastroenterol* 2011; **45**: 50-54 [PMID: 20717042 DOI: 10.1097/MCG.0b013e3181ec5c66]
111 **Chan JL**, Wong SL, Mantzoros CS. Pharmacokinetics of subcutaneous recombinant methionyl human leptin administration in healthy subjects in the fed and fasting states: regulation by gender and adiposity. *Clin Pharmacokinet* 2008; **47**: 753-764 [PMID: 18840030 DOI: 10.2165/00003088-200847110-00006]
112 **Lo KM**, Zhang J, Sun Y, Morelli B, Lan Y, Lauder S, Brunkhorst B, Webster G, Hallakou-Bozec S, Doaré L, Gillies SD. Engineering a pharmacologically superior form of leptin for the treatment of obesity. *Protein Eng Des Sel* 2005; **18**: 1-10 [PMID: 15790575 DOI: 10.1093/protein/gzh102]
113 **Zelissen PM**, Stenlof K, Lean ME, Fogteloo J, Keulen ET, Wilding J, Finer N, Rössner S, Lawrence E, Fletcher C, McCamish M. Effect of three treatment schedules of recombinant methionyl human leptin on body weight in obese adults: a randomized, placebo-controlled trial. *Diabetes Obes Metab* 2005; **7**: 755-761 [PMID: 16219020 DOI: 10.1111/j.1463-1326.2005.00468.x]
114 **Chitturi S**, Farrell G, Frost L, Kriketos A, Lin R, Fung C, Liddle C, Samarasinghe D, George J. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? *Hepatology* 2002; **36**: 403-409 [PMID: 12143049 DOI: 10.1053/jhep.2002.34738]
115 **Mantzoros CS**, Moschos S, Avramopoulos I, Kaklamani V, Liolios A, Doulgerakis DE, Griveas I, Katsilambros N, Flier JS. Leptin concentrations in relation to body mass index and the tumor necrosis factor-alpha system in humans. *J Clin Endocrinol Metab* 1997; **82**: 3408-3413 [PMID: 9329377 DOI: 10.1210/jcem.82.10.4323]
116 **Nakao K**, Nakata K, Ohtsubo N, Maeda M, Moriuchi T, Ichikawa T, Hamasaki K, Kato Y, Eguchi K, Yukawa K, Ishii N. Association between nonalcoholic fatty liver, markers of obesity, and serum leptin level in young adults. *Am J Gastroenterol* 2002; **97**: 1796-1801 [PMID: 12135038 DOI: 10.1111/j.1572-0241.2002.05846.x]
117 **Canbakan B**, Tahan V, Balci H, Hatemi I, Erer B, Ozbay G, Sut N, Hacibekiroglu M, Imeryuz N, Senturk H. Leptin in nonalcoholic fatty liver disease. *Ann Hepatol* 2008; **7**: 249-254 [PMID: 18753993]
118 **Serin E**, Ozer B, Gümürdülü Y, Kayaselçuk F, Kul K, Boyacioğlu S. Serum leptin level can be a negative marker of hepatocyte damage in nonalcoholic fatty liver. *J Gastroenterol* 2003; **38**: 471-476 [PMID: 12768390 DOI: 10.1007/s00535-002-1084-5]
119 **Procaccini C**, Galgani M, De Rosa V, Carbone F, La Rocca C, Ranucci G, Iorio R, Matarese G. Leptin: the prototypic adipocytokine and its role in NAFLD. *Curr Pharm Des* 2010; **16**: 1902-1912 [PMID: 20370676]
120 **Torres DM**, Harrison SA. Diagnosis and therapy of nonalcoholic steatohepatitis. *Gastroenterology* 2008; **134**: 1682-1698 [PMID: 18471547 DOI: 10.1053/j.gastro.2008.02.077]
121 **Lydatakis H**, Hager IP, Kostadelou E, Mpousmpoulas S, Pappas S, Diamantis I. Non-invasive markers to predict the liver fibrosis in non-alcoholic fatty liver disease. *Liver Int* 2006; **26**: 864-871 [PMID: 16911470 DOI: 10.1111/j.1478-3231.2006.01312.x]
122 **Fon Tacer K**, Kuzman D, Seliskar M, Pompon D, Rozman D. TNF-alpha interferes with lipid homeostasis and activates acute and proatherogenic processes. *Physiol Genomics* 2007; **31**: 216-227 [PMID: 17566076 DOI: 10.1152/physiolgenomics.00264.2006]

123 **Feingold KR**, Soued M, Serio MK, Adi S, Moser AH, Grunfeld C. The effect of diet on tumor necrosis factor stimulation of hepatic lipogenesis. *Metabolism* 1990; **39**: 623-632 [PMID: 2352479]

124 **Das SK**, Balakrishnan V. Role of cytokines in the pathogenesis of non-alcoholic Fatty liver disease. *Indian J Clin Biochem* 2011; **26**: 202-209 [PMID: 22468051 DOI: 10.1007/s12291-011-0121-7]

125 **Aigner E**, Theurl I, Theurl M, Lederer D, Haufe H, Dietze O, Strasser M, Datz C, Weiss G. Pathways underlying iron accumulation in human nonalcoholic fatty liver disease. *Am J Clin Nutr* 2008; **87**: 1374-1383 [PMID: 18469261]

126 **Bahcecioglu IH**, Yalniz M, Ataseven H, Ilhan N, Ozercan IH, Seckin D, Sahin K. Levels of serum hyaluronic acid, TNF-alpha and IL-8 in patients with nonalcoholic steatohepatitis. *Hepatogastroenterology* 2005; **52**: 1549-1553 [PMID: 16201116]

127 **Lebensztejn DM**, Kowalczuk D, Tarasów E, Skiba E, Kaczmarski M. Tumor necrosis factor alpha and its soluble receptors in obese children with NAFLD. *Adv Med Sci* 2010; **55**: 74-79 [PMID: 20371430 DOI: 10.2478/v10039-010-0008-5]

128 **Kugelmas M**, Hill DB, Vivian B, Marsano L, McClain CJ. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. *Hepatology* 2003; **38**: 413-419 [PMID: 12883485 DOI: 10.1053/jhep.2003.50]

129 **Ferreira VS**, Pernambuco RB, Lopes EP, Morais CN, Rodrigues MC, Arruda MJ, Silva LM, Vilar L. Frequency and risk factors associated with non-alcoholic fatty liver disease in patients with type 2 diabetes mellitus. *Arq Bras Endocrinol Metabol* 2010; **54**: 362-368 [PMID: 20625647 DOI: 10.1590/S0004-27302010000400004]

130 **Hui JM**, Farrell GC, Kench JG, George J. High sensitivity C-reactive protein values do not reliably predict the severity of histological changes in NAFLD. *Hepatology* 2004; **39**: 1458-1459 [PMID: 15122781 DOI: 10.1002/hep.20223]

131 **Zamora-Valdés D**, Méndez-Sánchez N. Experimental evidence of obstructive sleep apnea syndrome as a second hit accomplice in nonalcoholic steatohepatitis pathogenesis. *Ann Hepatol* 2007; **6**: 281-283 [PMID: 18007563]

132 **Abiru S**, Migita K, Maeda Y, Daikoku M, Ito M, Ohata K, Nagaoka S, Matsumoto T, Takii Y, Kusumoto K, Nakamura M, Komori A, Yano K, Yatsuhashi H, Eguchi K, Ishibashi H. Serum cytokine and soluble cytokine receptor levels in patients with non-alcoholic steatohepatitis. *Liver Int* 2006; **26**: 39-45 [PMID: 16420507 DOI: 10.1111/j.1478-3231.2005.01191.x]

133 **Yilmaz Y**, Kedrah AE, Ozdogan O. Cytokeratin-18 fragments and biomarkers of the metabolic syndrome in nonalcoholic steatohepatitis. *World J Gastroenterol* 2009; **15**: 4387-4391 [PMID: 19764089 DOI: 10.3748/wjg.15.4387]

134 **Wieckowska A**, Zein NN, Yerian LM, Lopez AR, McCullough AJ, Feldstein AE. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. *Hepatology* 2006; **44**: 27-33 [PMID: 16799979 DOI: 10.1002/hep.21223]

135 **Ulukaya E**, Yilmaztepe A, Akgoz S, Linder S, Karadag M. The levels of caspase-cleaved cytokeratin 18 are elevated in serum from patients with lung cancer and helpful to predict the survival. *Lung Cancer* 2007; **56**: 399-404 [PMID: 17316892 DOI: 10.1016/j.lungcan.2007.01.015]

136 **Tarantino G**, Conca P, Coppola A, Vecchione R, Di Minno G. Serum concentrations of the tissue polypeptide specific antigen in patients suffering from non-alcoholic steatohepatitis. *Eur J Clin Invest* 2007; **37**: 48-53 [PMID: 17181567 DOI: 10.1111/j.1365-2362.2007.01745.x]

137 **Wieckowska A**, Lopez AR, Zein NN, McCullough AJ, Feldstein AE. Noninvasive assessment of hepatocyte apoptosis in nonalcoholic fatty liver disease; a multi-center validation study. *Gastroenterology* 2007; **132**: A729 [DOI: 10.1002/hep.23050]

138 **Diab DL**, Yerian L, Schauer P, Kashyap SR, Lopez R, Hazen SL, Feldstein AE. Cytokeratin 18 fragment levels as a noninvasive biomarker for nonalcoholic steatohepatitis in bariatric surgery patients. *Clin Gastroenterol Hepatol* 2008; **6**: 1249-1254 [PMID: 18995215 DOI: 10.1016/j.cgh.2008.07.016]

139 **Musso G**, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011; **43**: 617-649 [PMID: 21039302 DOI: 10.3109/07853890.2010.518623]

140 **Malik R**, Chang M, Bhaskar K, Nasser I, Curry M, Schuppan D, Byrnes V, Afdhal N. The clinical utility of biomarkers and the nonalcoholic steatohepatitis CRN liver biopsy scoring system in patients with nonalcoholic fatty liver disease. *J Gastroenterol Hepatol* 2009; **24**: 564-568 [PMID: 19378390]

141 **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 [PMID: 15578508]

142 **Feldstein AE**, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. *Hepatology* 2009; **50**: 1072-1078 [PMID: 19585618]

143 **Sakugawa H**, Nakayoshi T, Kobashigawa K, Yamashiro T, Maeshiro T, Miyagi S, Shiroma J, Toyama A, Nakayoshi T, Kinjo F, Saito A. Clinical usefulness of biochemical markers of liver fibrosis in patients with nonalcoholic fatty liver disease. *World J Gastroenterol* 2005; **11**: 255-259 [PMID: 15633226]

144 **Santos VN**, Leite-Mór MM, Kondo M, Martins JR, Nader H, Lanzoni VP, Parise ER. Serum laminin, type IV collagen and hyaluronan as fibrosis markers in non-alcoholic fatty liver disease. *Braz J Med Biol Res* 2005; **38**: 747-753 [PMID: 15917956]

145 **Miele L**, Forgione A, La Torre G, Vero V, Cefalo C, Racco S, Vellone VG, Vecchio FM, Gasbarrini G, Rapaccini GL, Neuman MG, Grieco A. Serum levels of hyaluronic acid and tissue metalloproteinase inhibitor-1 combined with age predict the presence of nonalcoholic steatohepatitis in a pilot cohort of subjects with nonalcoholic fatty liver disease. *Transl Res* 2009; **154**: 194-201 [PMID: 19766963 DOI: 10.1016/j.trsl.2009.06.007]

146 **Preiss D**, Sattar N. Non-alcoholic fatty liver disease: an overview of prevalence, diagnosis, pathogenesis and treatment considerations. *Clin Sci* (Lond) 2008; **115**: 141-150 [PMID: 18662168 DOI: 10.1042/CS20070402]

147 **Dowman JK**, Tomlinson JW, Newsome PN. Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2011; **33**: 525-540 [PMID: 21198708 DOI: 10.1111/j.1365-2036.2010.04556.x]

148 **Lesmana CR**, Hasan I, Budihusodo U, Gani RA, Krisnuhoni E, Akbar N, Lesmana LA. Diagnostic value of a group of biochemical markers of liver fibrosis in patients with non-alcoholic steatohepatitis. *J Dig Dis* 2009; **10**: 201-206 [PMID: 19659788 DOI: 10.1111/j.1751-2980.2009.00386.x]

149 **Charlton M**, Angulo P, Chalasani N, Merriman R, Viker K, Charatcharoenwitthaya P, Sanderson S, Gawrieh S, Krishnan A, Lindor K. Low circulating levels of dehydroepiandrosterone in histologically advanced nonalcoholic fatty liver disease. *Hepatology* 2008; **47**: 484-492 [PMID: 18220286 DOI: 10.1002/hep.22063]

150 **Baulieu EE**, Thomas G, Legrain S, Lahlou N, Roger M, Debuire B, Faucounau V, Girard L, Hervy MP, Latour F, Leaud MC, Mokrane A, Pitti-Ferrandi H, Trivalle C, de Lacharrière O, Nouveau S, Rakoto-Arison B, Souberbielle JC, Raison J, Le Bouc Y, Raynaud A, Girerd X, Forette F. Dehydroepiandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge Study to a sociobiomedical issue. *Proc Natl Acad Sci USA* 2000; **97**: 4279-4284 [PMID: 10760294]

151 **Saruç M**, Yüceyar H, Ayhan S, Türkel N, Tuzcuoglu I, Can M. The association of dehydroepiandrosterone, obesity, waist-hip ratio and insulin resistance with fatty liver in postmenopausal women--a hyperinsulinemic euglycemic insulin clamp study. *Hepatogastroenterology* 2003; **50**: 771-774 [PMID: 12828082]

152 **Völzke H**, Aumann N, Krebs A, Nauck M, Steveling A, Lerch MM, Rosskopf D, Wallaschofski H. Hepatic steatosis is associated with low serum testosterone and high serum DHEAS levels in men. *Int J Androl* 2010; **33**: 45-53 [PMID: 19226405 DOI: 10.1111/j.1365-2605.2009.00953.x]

153 **Koehler E**, Swain J, Sanderson S, Krishnan A, Watt K, Charlton M.

Growth hormone, dehydroepiandrosterone and adiponectin levels in non-alcoholic steatohepatitis: an endocrine signature for advanced fibrosis in obese patients. *Liver Int* 2012; **32**: 279-286 [PMID: 22098614 DOI: 10.1111/j.1478-3231.2011.02637.x]

154 **Bahcecioglu IH**, Yalniz M, Ilhan N, Ataseven H, Ozercan IH. Levels of serum vitamin A, alpha-tocopherol and malondialdehyde in patients with non-alcoholic steatohepatitis: relationship with histopathologic severity. *Int J Clin Pract* 2005; **59**: 318-323 [PMID: 15857329 DOI: 10.1111/j.1742-1241.2004.00312.x]

155 **Osawa Y**, Hoshi M, Yasuda I, Saibara T, Moriwaki H, Kozawa O. Tumor necrosis factor-α promotes cholestasis-induced liver fibrosis in the mouse through tissue inhibitor of metalloproteinase-1 production in hepatic stellate cells. *PLoS One* 2013; **8**: e65251 [PMID: 23755201 DOI: 10.1371/journal.pone.0065251]

156 **Wieckowska A**, McCullough AJ, Feldstein AE. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: present and future. *Hepatology* 2007; **46**: 582-589 [PMID: 17661414 DOI: 10.1002/hep.21768]

157 **Romero FP**. Will non-invasive testing replace liver biopsy in the diagnosis and follow-up of non-alcoholic steatohepatitis (NASH)? *Rev Esp Enferm Dig* 2009; **101**: 521-527 [PMID: 19785490]

158 **Wieckowska A**, Feldstein AE. Diagnosis of nonalcoholic fatty liver disease: invasive versus noninvasive. *Semin Liver Dis* 2008; **28**: 386-395 [PMID: 18956295 DOI: 10.1055/s-0028-1091983]

**P- Reviewer**: Chan CH, Satapathy SK, Sugawara I **S- Editor**: Ji FF
**L- Editor**: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pathophysiology

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

World J Gastrointest Pathophysiol 2017 May 15; 8(2): 27-38

DOI: 10.4291/wjgp.v8.i2.27

ISSN 2150-5330 (online)

REVIEW

# Celiac disease: From pathophysiology to treatment

Ilaria Parzanese, Dorina Qehajaj, Federica Patrinicola, Merica Aralica, Maurizio Chiriva-Internati, Sanja Stifter, Luca Elli, Fabio Grizzi

Ilaria Parzanese, Luca Elli, Gastroenterology and Endoscopy Unit, Fondazione IRCCS Cà Granda, Ospedale Maggiore Policlinico, Department of Pathophysiology and Transplantation, University of Milan, 20122 Milano, Italy

Dorina Qehajaj, Federica Patrinicola, Fabio Grizzi, Department of Immunology and Inflammation, Humanitas Clinical and Research Center, 20089 Rozzano, Milan, Italy

Merica Aralica, Department of Laboratory Medicine, Clinical Hospital Center Rijeka, 51000 Rijeka, Croatia

Maurizio Chiriva-Internati, Division of Hematology and Oncology, Texas Tech University Health Sciences Center, Lubbock, TX 79430, United States

Sanja Stifter, Department of Pathology, School of Medicine, University of Rijeka, 51000 Rijeka, Croatia

Author contributions: Parzanese I, Qehajaj D, Patrinicola F, Aralica M, Chiriva-Internati M, Stifter S, Elli L and Grizzi F draft, discuss the manuscript and approved the final version.

Conflict-of-interest statement: The authors declare that they have no competing interests.



Manuscript source: Invited manuscript

Correspondence to: Dr. Fabio Grizzi, Department of Immunology and Inflammation, Humanitas Clinical and Research Center, Via Manzoni 56, 20089 Rozzano, Milan, Italy. fabio.grizzi@humanitasresearch.it
Telephone: +39-02-82245262
Fax: +39-02-82244590

Received: April 24, 2016
Peer-review started: April 25, 2016
First decision: June 6, 2016
Revised: March 8, 2017
Accepted: March 23, 2017
Article in press: March 24, 2017
Published online: May 15, 2017

## Abstract

Celiac disease, also known as "celiac sprue", is a chronic inflammatory disorder of the small intestine, produced by the ingestion of dietary gluten products in susceptible people. It is a multifactorial disease, including genetic and environmental factors. Environmental trigger is represented by gluten while the genetic predisposition has been identified in the major histocompatibility complex region. Celiac disease is not a rare disorder like previously thought, with a global prevalence around 1%. The reason of its under-recognition is mainly referable to the fact that about half of affected people do not have the classic gastrointestinal symptoms, but they present nonspecific manifestations of nutritional deficiency or have no symptoms at all. Here we review the most recent data concerning epidemiology, pathogenesis, clinical presentation, available diagnostic tests and therapeutic management of celiac disease.

Key words: Celiac disease; Epidemiology; Diagnosis; Treatment; Pathogenesis



**Core tip:** Celiac disease is a chronic inflammatory disorder of the small intestine, produced by the ingestion of dietary gluten products in susceptible people. It is a multifactorial disease, including genetic and environmental factors. Thanks to advanced understanding of its pathogenesis, numerous therapeutic strategies have been devised for the treatment of celiac

disease. But there is need of further basic research studies and randomized clinical trials to introduce them into usual management of this disease.

Parzanese I, Qehajaj D, Patrinicola F, Aralica M, Chiriva-Internati M, Stifter S, Elli L, Grizzi F. Celiac disease: From pathophysiology to treatment. *World J Gastrointest Pathophysiol* 2017; 8(2): 27-38 Available from: URL: http://www.wjgnet.com/2150-5330/full/v8/i2/27.htm DOI: http://dx.doi.org/10.4291/wjgp.v8.i2.27

## INTRODUCTION

Celiac disease defined an autoimmune disorder originating by an aberrant adaptive immune response against gluten-containing grains in susceptible individuals. Celiac disease was first described in 1888 by Samuel Gee, but only in 1953 it became clear the importance of the gluten in the origin of this pathology[1-3]. In celiac subjects the ingestion of gluten leads to an enteropathy with an impairment of the mucosal surface and, consequently, abnormal absorption of nutrients[4-7]. Celiac disease might be considered a syndrome, because of the wide spectrum of clinical manifestations and the involvement of various human systems. Celiac disease shows peculiar features in comparison to others autoimmune disorders, including the complete recovery of the mucosal damage as well as the reversibility of its progression and chronic dynamics, with a total avoidance of gluten. Conversely, it is now ascertained that undiagnosed celiac disease, might have severe consequences in children as well as in adult subjects[8-10]. Besides celiac disease and wheat allergy, a new entity has been included, apparently not driven by an immune response: The non-celiac gluten sensitivity (NCGS). The pathogenesis of NCGS remains largely unknown, although it is now ascertained that it includes a set of factors[3,11]. Here, we review the epidemiology, pathogenesis, clinical presentation, diagnostic tests and therapeutic management of celiac disease.

## EPIDEMIOLOGY

In the last decades a high number of epidemiological data have been reported. Nowadays celiac disease is one of the most frequent genetically based disorder in humans, although it was thought that some country, including United States, were exempt from this disease[12]. Europe is historically considered a geographical area at high frequency, with a prevalence of 1%-2%, although it has been recently shown a similar prevalence in United States[13,14]. Despite the advances in diagnosis, the overall prevalence of this disease remains still unclear. A variable frequency has been reported between European countries, although it is still uncertain whether it depends on the different screening tool, sample size or a real variability of celiac disease prevalence[15]. What is known is that many cases remains undiagnosed, as idealized with the "iceberg model" (Figure 1). Typical cases of celiac disease are diagnosed because of suggestive symptoms. The submerged part of the iceberg represents all the undiagnosed cases that usually show atypical, minimal, or even absent symptoms[16]. A multicenter study reported a prevalence of 1 out of 133 (0.75%) in healthy people in the United States, and similar frequency is confirmed by studies on European and Australian populations[12]. The overall prevalence of celiac disease ranges from 4.5% among high-risk subjects to 0.75% in not-at-risk subjects[12]. High-risk subjects include the relatives of patients with celiac disease, children or adults with celiac disease-associated symptoms (*i.e.*, diarrhea, abdominal pain and constipation) and children or adult subjects with celiac disease-associated disorders (*i.e.*, Diabetes Mellitus type-1, Down syndrome, anemia, infertility, osteoporosis)[17]. It has been shown that celiac disease is not exclusive of industrialized countries, but includes North Africa, Middle East and India with an incidence overlapping those of European countries[18-20]. However, given the worldwide distribution of the causal factors this heterogeneous diffusion is not surprising. It has been shown that the Saharawi, an Algerian population has the highest prevalence of celiac disease (nearly, 6%) among all of the worldwide populations[16,21,22].

## GENETIC SUSCEPTIBILITY

The best-characterized genetic risk factor for celiac disease, accounting for 35% of the total genetic risk, is the presence of genes encoding for MHC class II proteins including human leukocyte antigen (HLA) DQ 2 and HLA-DQ8[23-25]. Over 90% of affected subjects express HLA-DQ2 molecules; the remainder express HLA-DQ8. The frequency of celiac disease risk HLA genotypes is about 30%, whereas only 1%-3% develops the disease[26]. It is now accepted that HLA is one of the main but not sufficient factors involved in the onset of celiac disease, but a multitude of genetic factors are responsible in celiac disease susceptibility, as demonstrated by studies on monozygotic twins[27].

Recently, genome-wide association studies have identified 39 non-HLA loci that also predispose to celiac disease[28]. One of these genes may relate to genetic variants on chromosome 19, in the myosin IXB gene (*i.e.*, MYO9B), and may potentially predict responsiveness to a gluten-free diet (GFD)[29,30]. Both HLA-DQ2 and HLA-DQ8 codified for heterodimers located on Antigen-Presenting Cells (APCs)[31]. It has been ascertained that they present gluten peptides to antigen-specific T-lymphocytes in the intestinal mucosa, inducing their proliferation as well as cytokine production. In particular, tTG2 may transform non-charged glutamine into negatively charged glutamic acid[32].

### *Environmental factors*

Feeding patterns in the first year of life and potential

viral infections (*i.e.*, rotavirus)[33] might be involved in the development of celiac disease[34]. A prospective study has investigated the role of a specific infectious agent in celiac disease and the authors have found that an increased frequency of rotavirus infection predicts increased risk of celiac disease autoimmunity in children[35]. Gluten, one of the most common ingredients in human nutrition[36], is mainly composed by prolamines and glutenin. The prolamines in wheat are gliadins, in rye secalines and in barley hordeins. Catassi *et al*[37] proposed that 50 mg gluten/day is the minimum amount able to determine evident alterations to the small-intestinal mucosa in celiac disease subjects. Others pivotal "environmental factors" as the milk-feeding type and the duration of breast-feeding, can also play a role, influencing the intestinal microenvironment[38]. In addition, increased intestinal Gram-negative and reduced Bifidobacteria, has been found in celiac disease subjects[39]. Other debated environmental factors could be represented by heavy metals[40] and bacterial TG present in food stuff[41].

## IMMUNE SYSTEM AND THE CELIAC DISEASE

Celiac disease resembles a systemic immune-mediated disorder[34,42-44]. The primary mechanism involved in celiac disease is related to an inappropriate adaptive immune response to gluten-derived peptides. It has been ascertained that prolamines contain critical epitopes presented by either HLA-DQ2 or HLA-DQ8 induce a $CD4^+$ T-lymphocytes response. In celiac disease pathogenesis the role exerted by the intestinal epithelia barrier, physiologically impermeable to macromolecules such as gliadin is actually recognized. In people with a genetic susceptibility to develop celiac disease, gliadin interacts with the intestinal cells to trigger the disassembling of the inter-enterocyte tight junctions (TJs). The impairment of the TJs determines the up-regulation of zonulin, a peptide involved in TJ regulation and responsible for the increased gut permeability. Gliadin peptides pass through the epithelial barrier and activate T-lymphocytes located in the lamina propria. Activated $CD4^+$ T-lymphocytes produce high levels of pro-inflammatory cytokines, inducing either a T-helper 1 pattern dominated by IFN-γ, and a T-helper 2 pattern, which causes a clonal expansion of B-lymphocytes that subsequently differentiate in plasma-cells secreting anti-gliadin and anti-tissue-transglutaminase antibodies[45]. Some gliadin peptides that are not recognized by T-lymphocytes activate both APCs and intestinal epithelial cells; in particular, $CD8^+$ T-lymphocytes may be stimulated by interleukin (IL)-15. An increased density of $CD8^+$ intraepithelial cells is considered as a hallmark of celiac disease[34]. Gliadin-specific T-cell responses have been found to be enhanced by the action of tissue transglutaminase, an enzyme located in the extracellular space of the sub-epithelial region or at the epithelial brush border[46].

## HISTOLOGICAL FEATURES OF THE CELIAC DISEASE

Physiologically the height of enterocytes ranges between 29-34 μm. T-cells are usually located in the *lamina propria*; however, the number of intraepithelial lymphocytes (IELs) is highly variable. A large number of subjects without disease have less than 20 lymphocytes per 100 epithelial cells; based on the experiences of Hayat *et al*[47] and Veress *et al*[48], a density of IELs between 25 and 29/100 epithelial cells is considered borderline and pathological over 30/100 epithelial cells. Tissue samples taken from subjects affected by celiac disease mainly show: (1) Decreased enterocyte height; (2) Crypt hyperplasia; (3) Villous atrophy; (4) Increased intraepithelial T lymphocytes.

Although still debated, recently, Mubarak *et al*[49] have shown that staining for CD3 has an additional value in the histological detection of celiac disease lesions[49,50]. Immunohistochemical stains for CD3 and CD8 do not improve detection of gluten-sensitive enteropathy in duodenal biopsies. A different grading has been proposed for the evaluation of the gluten free diet in duodenal mucosal healing[51].

Table 1 reports Marsh classification of histologic findings in celiac disease[52]. Modifications to this scoring system have been proposed[53,54]. Oberhuber *et al*[55] suggested that Marsh Ⅲ lesions should be included into *a*, *b*, and *c* categories. However, Figure 2 shows two prototypical tissues classified as Marsh Ⅱ (A) and Marsh ⅢA (B).

## CELIAC DISEASE: CLINICAL PRESENTATION

Celiac disease is greatly heterogeneous, at least in part depending on the patient's age, the duration and extent of disease, and the presence of extra-intestinal comorbidities.

Although celiac disease was originally thought as a pediatric disorder, the diagnosis is increasingly made in adults[16]. Various subtypes of celiac disease have been described[56].

Classical or typical form. It is characterized by common clinical symptoms related to abnormal intestinal absorption. Generally occurs between 6 and 18 mo of age, after the introduction of weaning foods containing prolamines[57]. Histology shows villous atrophy and crypt hyperplasia.

### *Atypical form*

It is characterized by a prevalence of extra-intestinal symptoms with few or no gastrointestinal symptoms. Usually, atypical forms are encountered in older children and adults and the common features of abnormal

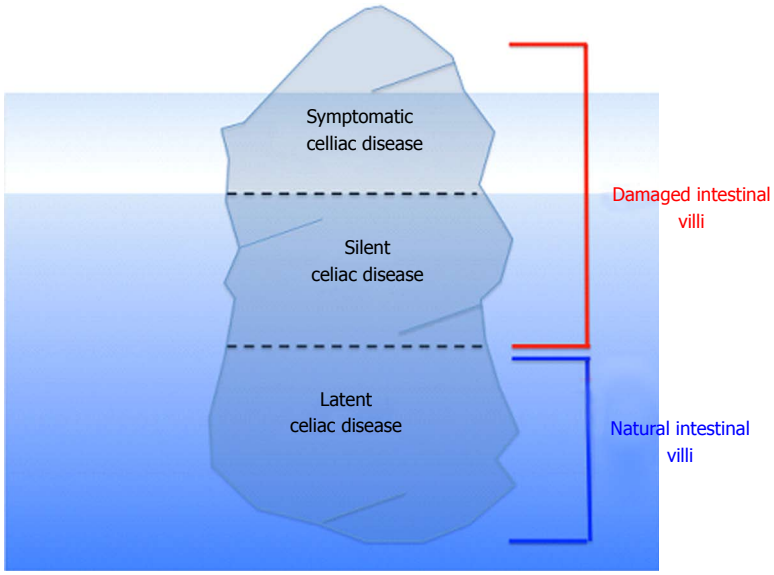


**Figure 1 The "iceberg model" idealizing the interplay between celiac disease genetic makeup and exposure to gluten, the environmental trigger of the disease.**

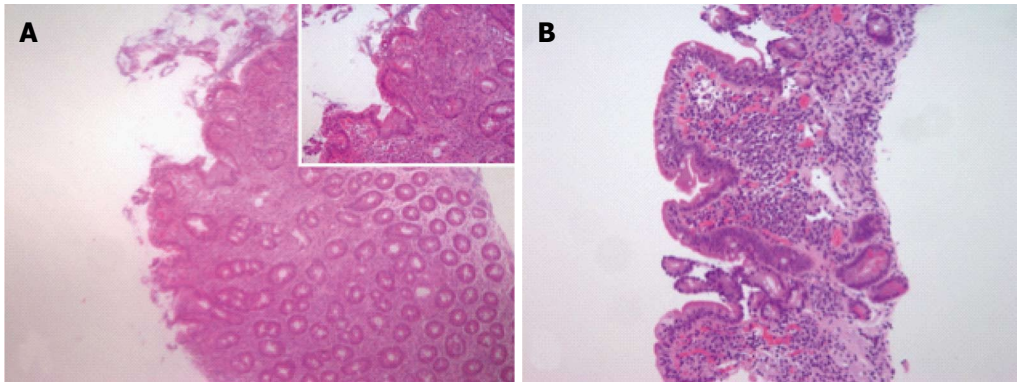


**Figure 2 Histological features of celiac disease.** A: Example of tissue scored as Marsh 2 characterized by lymphocytic enteritis with crypt hyperplasia: Intraepithelial lymphocytosis and elongation and branching of crypts in which there is an increased proliferation of epithelial cells; B: Example of tissue scored as Marsh 3A characterized by partial villous atrophy, the villi are blunt and shortened. Arbitrarily, samples are classified as partial villous atrophy if the villus-crypt ratio was less than 1:1 (Objective magnification × 4, inset × 10).

**Table 1 Marsh classification of histologic findings in celiac disease**

| | |
|---|---|
| Marsh 0 | Normal mucosal architecture without significant intraepithelial lymphocytic infiltration. |
| Marsh I | Lymphocytic enteritis: Normal mucosal architecture with a marked infiltration of villous epithelium by lymphocytes; arbitrarily defined marked as more than 30 lymphocytes per 100 enterocytes |
| Marsh II | Lymphocytic enteritis with crypt hyperplasia: intraepithelial lymphocytosis and elongation and branching of crypts in which there is an increased proliferation of epithelial cells |
| Marsh III | Intraepithelial lymphocytosis, crypt hyperplasia, and villous atrophy. There are 3 distinct stages of villous atrophy |
| Marsh IIIA | Partial villous atrophy, the villi are blunt and shortened. Arbitrarily, samples are classified as partial villous atrophy if the villus-crypt ratio was less than 1:1 |
| Marsh IIIB | Subtotal villous atrophy, villi are clearly atrophic, but still recognizable |
| Marsh IIIC | Total villous atrophy, villi are rudimentary or absent, and the mucosa resemble colonic mucosa. |

Modifications to this scoring system have been proposed[53,54]. Oberhuber *et al*[55] suggested that Marsh III lesions should be included into a, b, and c categories. However, Marsh *et al*[52] examined these subdivisions by means of correlative light and scanning electron microscopy, and demonstrate that Oberhuber's classification is untenable. In their view, this categorization reflects misinterpretations of the real architectural contours of flat mucosae.

absorption are absent.

### *Silent or asymptomatic form*

It is characterized by serological and histological abnormalities without evidence of clinical symptoms. This subtype is often observed in subjects with a family history of celiac disease, patients with associated autoimmune (*i.e.*, type 1 diabetes) or genetic disorders

(*i.e.*, Down, Turner, or Williams syndrome).

### *Latent form*

It is characteristic of subjects with previous asymptomatic celiac disease, although a gluten-containing diet. Positive serology but no villous atrophy or others tissue abnormalities are recognized. Troncone *et al*[58] postulated that the presence of elevated endomysial antibodies in these patients might be one of the most important predictor of progression.

### *Potential form*

The term "potential" is used in individuals who have never had diagnosis of celiac disease, but show presence of appropriate genetic background (HLA-DQ2/DQ8), positive serology, with normal or mildly abnormal histology.

### *Refractory form*

It is defined by the presence of malabsorptive symptoms and villous atrophy that persist 1 year after a strict gluten-free diet. Several refractory patients (roughly, 5%-30%) never respond to a gluten-free diet[59], others initially responded but have a recurrence of symptoms and intestinal damage. Two different subtypes of refractory celiac disease have been recognized: "Type 1", showing a normal intraepithelial lymphocytes count and "type 2" presenting aberrant intraepithelial lymphocytes[29].

Celiac disease can affect individuals from any age, but two peaks can be seen: In the childhood (< 6-year-old) and 4$^{th}$-5$^{th}$ decade[46]. Classical presentation is more frequent in pediatric and tends to occur early in life (6-24 mo), whereas atypical presentation generally occurs at a later age (> 5-year-old) and in adults[60].

## DERMATOLOGICAL CELIAC DISEASE

Dermatitis herpetiformis is an inflammatory cutaneous disease, presenting with diffuse, symmetrical, polymorphic lesions consisting of erythema, urticarial plaques, papules, herpetiform vesiculae and blisters followed by erosions, excoriations and hyperpigmentation. It is characterized by typical histopathological and immunopathological findings. Rarely it is diagnosed in childhood but commonly appears in the third decade.

## MAIN CONDITIONS ASSOCIATED TO CELIAC DISEASE

A set of conditions can be associated with celiac disease. The term "associated conditions" refers to states that are found more frequently in patients affected by celiac disease[61]. These conditions include "genetic disorders" such as Down syndrome, Turner syndrome and Williams syndrome, and "autoimmune" or "neurological" disorders.

### *Type 1-diabetes*

One of the most recognized and widely investigated disorders associated with celiac disease is type 1-diabetes[62]. Ludvigsson *et al*[63] reported that type 1-diabetes constitutes a 5- to 10-fold risk increase for celiac disease in a very large cohort of children. This increasing of risk may partly be explained by shared genetic risk represented by HLA. A percentage approximately of 5%-10% of patients affected by type 1-diabetes presented celiac disease related antibodies with up to 75% having abnormalities on small intestinal biopsy tissue[64]. The prevalence of celiac disease ranges between 1% to 19% in patients with type 1 diabetes mellitus[65]. Prospective studies of high-risk infants for type 1 diabetes and celiac disease have been shown that early introduction of gluten is associated with an increased risk for autoimmunity[66-68]. However, the relationship between the two conditions is still debated[69].

### *Autoimmune thyroid disorders*

In patients affected by celiac disease it has been reported an increased prevalence (nearly, 2%-5%) of thyroid disorders (*i.e.*, hyperthyroidism-Graves's disease or hypothyroidism-Hashimoto's thyroiditis), diagnosed either before than after the diagnosis of gluten-enteropathy[65]. These two conditions share genetic risk factors represented by HLA-DQ2 and DQ8. HLA-DQ2 and DQ8 haplotypes have been association with Hashimoto's thyroiditis, while HLA-DQ2 association is less clear in Graves' disease. This difference between hyper- and hypothyroidism is reflected also by the greater risk of celiac disease in patients with Hashimoto's disorder than patients with Graves[70]. In addition to HLA, it has been reported an association with the gene encoding cytotoxic T-lymphocyte-associated antigen-4[65]. Another mechanism related to the association between these two conditions is represented by abnormal absorption with consequent selenium deficiency induced by celiac disease. Stazi *et al*[70] highlighted that the abnormal selenium absorption in celiac disease could be the factor directly leading to thyroid and intestinal damage, since thyroid is particularly sensitive to selenium deficiency. In the setting of autoimmune thyroid disease it should be useful to pay attention to celiac disease marker and to monitor growth and pubertal status. In patients with celiac disease, a screening for thyroid abnormalities has been also suggested in some cases[61].

### *Autoimmune hepatitis and other forms of liver involvement*

The involvement of liver is common among patients affected by celiac disease[71]. Hypertransaminasemia has been reported in about 40% of adults and in 54% of children with a classical presentation of celiac disease at the time of diagnosis[72]. Conversely, celiac disease is present in about 9% of patients with chronic unexplained hypertransaminasemia[73,74]. It has been

postulated that the mechanism leading to hepatic damage is related to entry of toxins, inflammatory molecules and antigens in the portal circulation. Volta *et al*[75] pointed up about the need of a serological screening for celiac disease in all patients with persistent hypertransaminasemia of unknown cause. Moreover, exclusion of a gluten-related liver damage is necessary for all patients affected by autoimmune liver disorders or by those forms of severe liver disease, whose etiology remains unknown, and for patients enrolled for liver transplant. A prolonged exposition to gluten in a patient with an overlooked celiac disease, in fact, can causes chronic hepatitis and liver cirrhosis[75,76].

### *Neurological disorders*

It has been reported a potential link between celiac disease and different neurological disorders[77]. Data concerning the association between neurological conditions and celiac disease remain poor. Although ataxia is a neurological disorder indicated in some patients with celiac disease[78], the most frequent neurologic condition in celiac disease subjects is epilepsy, showing a prevalence between 1.2% and 5%[79]. Its clinical spectrum in association with celiac disease varies from focal to generalize with variable outcome and response to gluten avoidance. In 1985 it has been described a more specific and rare syndrome characterized by the co-presence of celiac disease, epilepsy, and occipital calcifications (CEC). Gobbi suggested that the HLA genotype predisposing to CEC is the same to that predispose to celiac disease[79].

## COMPLICATIONS ASSOCIATED WITH UNTREATED CELIAC DISEASE

Evidences that celiac disease in adults, especially if diagnosed late is burdened by complications have been reported. Among them: (1) Osteoporosis: It represents the most common complication resulting from abnormal calcium absorption secondary to defective calcium transport by the diseased small intestine, but also due to vitamin D deficiency. With an early gluten free diet in children it is possible to prevent the bone disease in adult life; (2) Enteropathy-associated intestinal T cells lymphoma: It represents one of the most important complication; (3) Collagenous sprue: Patients do not react to diet and histology shows extra-cellular matrix components in the intestinal wall at the level of the superficial sub-epithelial layer. This morphological pattern is very similar to the condition of collagenous colitis described in the colon, where the thickness of the connective band best highlighted with Masson's trichrome is more than 15 nanometers, although this is a very rare event described in the literature; (4) Refractory sprue: This condition is depicted as collagenous sprue although can be identified by immunohistochemical staining, demonstrating that T lymphocytes, which in normal conditions express CD3 and CD8, in this case present only the expression of CD3 and not of CD8; (5) Ulcerative jejunoileitis: Extensive ulceration of the intestinal mucosa, and involving ileum and jejunum. It presents around 50 years old with chronic diarrhea, steatorrhoea and complications of intestinal ulceration (perforation, haemorrhage or obstruction)[59]; (6) Non-Hodgkin lymphoma: Recent data suggests an association between celiac disease and Non-Hodgkin lymphoma[80]; (7) Small bowel adenocarcinoma: Even rare, a connection between carcinoma of the small bowel and celiac disease is known since 1958[81]. The etiologic factors predisposing to malignancy in celiac disease are uncertain. Possibilities include immunologic disturbance associated with mucosal lymphocyte infiltration, pre-malignant changes in the damaged surface epithelial cells, increased permeability to oncogenic factors and abnormal absorption of protective substances such as vitamins A and E; and (8) Reproductive disorders: Celiac disease might be associated with decreased fertility in both males and females[82].

## DIAGNOSTIC TESTS OF CELIAC DISEASE

### *Serological tests*

**Anti-tissue transglutaminase antibodies:** the best strategy for serological diagnosis is the blood detection of IgA anti-tissue transglutaminase antibodies (tTGA) by enzyme-linked immunosorbent assay (ELISA). These antibodies show a sensitivity up to 97%, a specificity around 96%, and an accuracy of 98%, whereas IgA anti-endomysial (IgA EMA) antibodies are employed as a confirmatory test in tTGA positive cases due to their higher specificity (about 100% *vs* 91% of tTGA). In concomitance of IgA deficiency and celiac disease, found in around 2%-10% of the patients, it is recommended to detect celiac disease testing tTG-IgG. "False negative" occurs, as previously reported, in case of IgA deficiency. The IgA EMA represents the most specific test (approximately 100%), with a sensitivity around 94% and a diagnostic accuracy of 97%[83]. However, EMA are routinely detected by indirect subjective immunofluorescence[84]. These antibodies can also result falsely negative in case of IgA deficiency and in children aged > 2 years.

**Anti-gliadin antibodies:** The antigliadin (AGA) antibodies (IgG and IgA) are today no longer recommended because of their low sensitivity and specificity and inferior accuracy, except in younger children[85].

**Deamidated gliadin peptides:** Actually detection of antigliadin antibodies have been replaced by the more recently developed immunoassays employing antibodies to deamidated gliadin peptides, IgA and IgG. To increase the diagnostic accuracy, in the last years the clinicians tend to prescribe serial testing.

### *Histology*

The gold standard to diagnose celiac disease in adulthood

is the intestinal biopsy sampled by endoscopy[54,86]. Histology of celiac disease consists of an integrated assessment of different entities: villous atrophy, crypt hyperplasia, decreased enterocyte height, inflammatory infiltrates in small-bowel mucosal biopsies. Based on one or more of these elementary lesions the histopathology of celiac disease is subdivided into different diagnostic categories according to the Marsh classification[86] (Table 1).

The open question remains the early lesions, *i.e.*, normal villi with a pathologic increase in intraepithelial T-lymphocytes, that it is possible to find in many others conditions[87]. Villanacci *et al*[86] pointed out that three conditions deserve specific mention: (1) "Autoimmune enteritis"; (2) Damage by drugs: especially non-steroidal anti-inflammatory drugs (NSAID) that cause morphological modifications likely to those of celiac disease; and (3) The co-infection with *Helicobacter pylori* in the stomach[86].

Recently, have been introduced methods to quantify pathologic features of the small intestinal mucosa in celiac patients[88].

### *Genetic analyses*

HLA testing should not be routinely performed in all celiac disease cases, but it is indicated only when diagnosis is controversial. Large multicenter studies have shown that only 0.4% of celiac disease patients are both DQ2 and DQ8 negative[89]. In the absence of HLADQ2/8, it is consented to rule out predisposition to celiac disease in family members of celiac patients[86]. HLA test represents a helpful tool particularly for potential celiac disease, to suggest (if positive) or reject (if negative) the diagnosis. Furthermore, HLA negativity in patients with villous atrophy and negative serology should direct towards other possible causes of these histological alterations. Many disorders, such as lactose intolerance, bacterial overgrowth, Crohn's disease, infectious diseases (*i.e.*, Giardia lamblia, Cryptosporidium, Microsporidium, Cytomegalovirus, Herpes virus, Whipple's disease), characterized by malabsorption share the same histological findings as celiac disease.

### *In vitro gluten challenge test*

Currently, several studies have proposed that gluten-sensitive immunological activation in celiac disease can be reproduced by *in vitro* gluten challenge test using culture cells from the duodenal mucosa[90].

## CURRENT TREATMENTS OF CELIAC DISEASE

### *Life-long gluten-free diet*

The current available treatment for celiac disease is life-long gluten-free diet[91-93]. Generally clinical improvement is achieved within a few weeks and the mucosal damage recovers in 1-2 years[16]. As patients with celiac disease may have accompanying brush border lactase deficiency secondary to damage to surface epithelial cells, milk and dairy products should be avoided in the first period of treatment too. As vitamin B deficiency is common after an extended period on a GFD, all patients are advised to take a gluten-free multivitamin[84]. Early diagnosis and treatment are fundamental in pediatric celiac disease particularly, as some of the complications may be irreversible: Growth retardation, abnormal dentition, osteoporosis[29]. Several observational studies suggest that prolonging breast-feeding and delaying and gradually introducing gluten in the first year of life may reduce the risk of celiac disease development during childhood[60]. As described above, even products specifically targeted to dietary treatment of celiac disease may contain tiny amounts of gluten proteins[16]. Another cause of uncertainty is represented by the differences in the labeling rules for food products existing among countries. Specific considerations about gluten free diet and refractory celiac disease should be taken. Even with respect of dietary restriction, actually, in the minority of patients affected by refractory celiac disease (RCD), GFD is ineffective. It has showed a higher mortality compared with RCD type 1, explainable by the more severe malnutrition combined with the higher risk of developing overt lymphoma[94]. These forms of the disease can require corticosteroids and other immunosuppressant, like azathioprine or cyclosporin, which can improve transiently clinical symptoms in most patients. But till date, it has not yet been possible to design effective treatments for the both form of RCD[94]. Furthermore, it is important remember that these drugs may enhance the risk of progression into an overt T-cell lymphoma, so they require caution particularly in RCD type 2 patients, at risk of developing this complication. Recently, have been used with some success chemotherapy agents such as the anti-T cell nucleoside analogues Cladribine and Pentostatine, and it has been proposed also as a therapeutic option the stem cell transplantation[95,96].

Other drug-based therapies are in under investigations although patients indicate GFD as a good and well-tolerated therapy[97,98].

### *Gluten-degrading enzymes*

Enzyme supplement therapy with bacterial prolyl-endopeptidasis expressed by various microorganism has been proposed to accelerate gluten digestion in the gastrointestinal tract and thus to destroy T cell epitopes[99]. Prolyl-endopeptidasis are proline-specific enzymes capable to cleave gluten peptides. Actually, there are introduced into clinical trials two drug candidates, ALV003 and AN-PEP (Aspergillus niger prolyl-endoprotease). Recent data on results of two phase 1 clinical trials have revealed that pre-treating gluten with ALV003 eliminates the peripheral blood T cell response in celiac disease patients, suggesting the potential therapeutic utility of gluten-specific enzymes to treat celiac disease[100]. Currently is undergoing clinical phase Ⅱa testing showing a significantly reduce gluten-

related T-cell responses compared with placebo but without a significantly reduction of symptoms typically induced by the gluten[101].

AN-PEP is an enzyme that degrades gluten peptides efficiently in a pH compatible with that found in the stomach. Therefore this enzyme might be suitable for oral supplementation but further studies are necessary[102].

### *Modified grains*

Can be developed either through selective breeding of early wheat species or using small interfering RNA (siRNA) technology to mutate or silence immunostimulatory sequence[103].

### *Blocking gluten entry across the intestinal epithelium*

Zonulin inhibitor larazotide (AT-1001) corrects intestinal barrier defects. It has been explored in an animal model[99]. AT-1001 is currently the best-studied pharmacologic agent to treat patients with celiac disease, actually undergone in phase II clinical trials[104]. It has been shown that patients treated with AT-1001 had an improved symptom score, a less pronounced autoantibody response and pro-inflammatory production, and lower urinary nitrate excretion when compared with the placebo controls[105].

### *Rho/Rho kinase inhibition*

It has been clarified that the increase in intestinal permeability is dependent on Rho kinase (ROCK) activity[106]. In addition to regulating tight junction structure and function, ROCK is known to regulate axon growth[107,108]. The drug could be used to establish whether ROCK inhibition can reverse gluten-dependent increase in intestinal permeability in these patients[104].

### *Immunotherapy*

The first observation of the occurrence of celiac disease following allogenic bone marrow transplantation performed in a patient with acute leukemia, made evident the involvement of T-lymphocyte in pathogenesis of this condition[109]. Since then, a lot of acknowledgments have been acquired such that many efforts are being made to develop immunologic therapeutic tools. Cytokine therapies based either on amplification of regulatory cytokines or on blockage of inflammatory cytokines expression are largely diffused for management of the severe autoimmune disorders. Also for celiac disease resistant to dietary approach and especially for refractory celiac disease, the use of immunomodulators is developing. IL-15 blocking antibodies have shown the capability to induce intra-epithelial lymphocytes apoptosis in the intestinal epithelium of human IL-15 transgenic mouse models[110]. These antibodies have been only investigated on patients with rheumatoid arthritis and a human study for celiac disease is still awaited. Only human recombinant IL-10 was tried and it showed suppression of gluten-dependent T-cell activation in celiac disease cultured intestinal mucosa[111]. But later, a pilot study conducted on patients affected by refractory celiac disease did not show any pharmacological efficacy of this monoclonal antibody[110]. Anti-IFN-γ antibodies demonstrated a good tolerance among patients with inflammatory bowel disease, but the use in patients with celiac disease needs of more investigations. Equal considerations can be assumed also for the use of antibodies anti-TNF-α, commonly recognized for the treatment of inflammatory bowel disease but described only in few case reports of patients with refractory celiac disease[112,113]. Another possible target of immunotherapy is represented by chemokines and their receptors, which play a significant role in the pathogenesis of celiac disease through the recruitment of lymphocytes in the gut. T-lymphocytes that home to the small intestine express CCR9, which binds to the CCL25 secreted by intestinal epithelial cells, and integrin $\alpha_4\beta_7$, which binds to mucosal vascular addressin cell 1 (MAdCAM-1). Therefore both molecules are necessary for migration of T-cells to the intestinal mucosa and could represent potential therapeutic targets. Furthermore, a monoclonal antibody anti-integrin $\alpha_4$ (Natalizumab) has revealed effectiveness in Crohn's disease suggesting a possible effectiveness also in celiac disease[114] as well as a CCR9 inhibitor (CCX282-B) and integrin $\alpha_4\beta_7$ blocking antibody (LDP-02), both already studied in patients affected by Crohn's disease but still under investigation for celiac disease[115]. Some concern could be raised about the benefit of blocking lymphocyte homing to the intestine, and potential long-term adverse consequences, also because beneficial immunosuppressive regulatory T cells are equally inhibited. Since its demonstration, the possibility to develop tolerance to gluten in certain patients affected by celiac disease has gained more attractiveness as another potential immunomodulatory approach[116,117]. An alternative method studied for induction of tolerance to gluten is oral administration of a genetically modified Lactococus lactis bacterium, capable of secreting deamidated DQ8-restricted gliadin epitope. This experiment was conducted on previously sensitized transgenic mice and demonstrated the induction of $Foxp3^+$ regulatory T-lymphocytes and a significant suppression of local and systemic T-cell responses to the corresponding gliadin peptide[104,118]. Also this approach still needs of human trials, although others clinical trials for non-specific immunomodulation have been carried out to test the role of parasitic infection in inhibition of immune response to gluten[119]. In fact, the disappearance of intestinal parasites from humans in developed countries has created a predisposition to develop autoimmune disease, a phase I clinical trial using larvae from the hookworm Necator Americanus has been initiated with the purpose of establishing a potential shift of T-cells response toward a suppressive T-regulatory response, however to date, any significant effect was revealed by this infection on gluten-induced enteropathy[120].

### *Vaccines*

Clinical trials have been started with a prototypical vaccine based on a set of gluten peptides that are recognized by HLA-DQ2 in an immunodominant manner[104], and phase I has been recently completed. A great interest is focused on vaccination, especially for raising the compliance of many patients which could beneficiate with a single-dose administration rather than a daily intake of other treatment options[110]. The risk of immune system activation related to the vaccine therapy and its side effects represent a still open question that need of further investigations. There is still need of more studies also for the most part of the previously mentioned alternative treatment options, with large long-term clinical trials that could answer to the unresolved questions relating to their real clinical effectiveness, their safety, and their affordability.

## CONCLUSION

It is indubitable that celiac disease remains a still controversial and complex human disorder. There is need of further research studies and randomized clinical trials to introduce them into usual management of this disease. A number of unanswered questions remain to clarify including the real associations of celiac disease with other conditions, and the impact of some environmental factor in its pathogenesis. There are still controversial opinion about the effective role of the breast feeding in celiac disease, what is to be elucidated if it could offer a permanent protection or if only delays the appearance of the disease. In addition, an improvement in non-invasive diagnostic tests could allow avoidance of endoscopy, especially in pediatric patients, and a more defined efficacy of the new therapeutic tools could improve quality of life either in term of reduction of complications and physical health either in term of social life.

## REFERENCES


1 **Losowsky MS**. A history of coeliac disease. *Dig Dis* 2008; **26**: 112-120 [PMID: 18431060 DOI: 10.1159/000116768]

2 **Dicke WK**, Weijers HA, Van de kamer JH. Coeliac disease. II. The presence in wheat of a factor having a deleterious effect in cases of coeliac disease. *Acta Paediatr* 1953; **42**: 34-42 [PMID: 13050382]

3 **Elli L**, Branchi F, Tomba C, Villalta D, Norsa L, Ferretti F, Roncoroni L, Bardella MT. Diagnosis of gluten related disorders: Celiac disease, wheat allergy and non-celiac gluten sensitivity. *World J Gastroenterol* 2015; **21**: 7110-7119 [PMID: 26109797 DOI: 10.3748/wjg.v21.i23.7110]

4 **Fasano A**, Catassi C. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. *Gastroenterology* 2001; **120**: 636-651 [PMID: 11179241]

5 **Green PH**, Lebwohl B, Greywoode R. Celiac disease. *J Allergy Clin Immunol* 2015; **135**: 1099-1106; quiz 1107 [PMID: 25956012 DOI: 10.1016/j.jaci.2015.01.044]

6 **Assimakopoulos SF**, Papageorgiou I, Charonis A. Enterocytes' tight junctions: From molecules to diseases. *World J Gastrointest Pathophysiol* 2011; **2**: 123-137 [PMID: 22184542 DOI: 10.4291/wjgp.v2.i6.123]

7 **Jeon MK**, Klaus C, Kaemmerer E, Gassler N. Intestinal barrier: Molecular pathways and modifiers. *World J Gastrointest Pathophysiol* 2013; **4**: 94-99 [PMID: 24244877 DOI: 10.4291/wjgp.v4.i4.94]

8 **Rubio-Tapia A**, Kyle RA, Kaplan EL, Johnson DR, Page W, Erdtmann F, Brantner TL, Kim WR, Phelps TK, Lahr BD, Zinsmeister AR, Melton LJ, Murray JA. Increased prevalence and mortality in undiagnosed celiac disease. *Gastroenterology* 2009; **137**: 88-93 [PMID: 19362553 DOI: 10.1053/j.gastro.2009.03.059]

9 **Holmes S**. Coeliac disease: symptoms, complications and patient support. *Nurs Stand* 2010; **24**: 505-506: quiz 508 [PMID: 20509383]

10 **Norström F**, Lindholm L, Sandström O, Nordyke K, Ivarsson A. Delay to celiac disease diagnosis and its implications for health-related quality of life. *BMC Gastroenterol* 2011; **11**: 118 [PMID: 22060243 DOI: 10.1186/1471-230X-11-118]

11 **Elli L**, Roncoroni L, Bardella MT. Non-celiac gluten sensitivity: Time for sifting the grain. *World J Gastroenterol* 2015; **21**: 8221-8226 [PMID: 26217073 DOI: 10.3748/wjg.v21.i27.8221]

12 **Fasano A**, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, Elitsur Y, Green PH, Guandalini S, Hill ID, Pietzak M, Ventura A, Thorpe M, Kryszak D, Fornaroli F, Wasserman SS, Murray JA, Horvath K. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Arch Intern Med* 2003; **163**: 286-292 [PMID: 12578508]

13 **Rewers M**. Epidemiology of celiac disease: what are the prevalence, incidence, and progression of celiac disease? *Gastroenterology* 2005; **128**: S47-S51 [PMID: 15825126]

14 **Fasano A**. Where have all the American celiacs gone? *Acta Paediatr Suppl* 1996; **412**: 20-24 [PMID: 8783750]

15 **Mustalahti K**, Catassi C, Reunanen A, Fabiani E, Heier M, McMillan S, Murray L, Metzger MH, Gasparin M, Bravi E, Mäki M. The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. *Ann Med* 2010; **42**: 587-595 [PMID: 21070098 DOI: 10.3109/07853890.2010.505931]

16 **Lionetti E**, Catassi C. New clues in celiac disease epidemiology, pathogenesis, clinical manifestations, and treatment. *Int Rev Immunol* 2011; **30**: 219-231 [PMID: 21787227 DOI: 10.3109/08830185.2011.602443]

17 **Vriezinga SL**, Schweizer JJ, Koning F, Mearin ML. Coeliac disease and gluten-related disorders in childhood. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 527-536 [PMID: 26100369 DOI: 10.1038/nrgastro.2015.98]

18 **Cataldo F**, Montalto G. Celiac disease in the developing countries: a new and challenging public health problem. *World J Gastroenterol* 2007; **13**: 2153-2159 [PMID: 17465493]

19 **Barada K**, Bitar A, Mokadem MA, Hashash JG, Green P. Celiac disease in Middle Eastern and North African countries: a new burden? *World J Gastroenterol* 2010; **16**: 1449-1457 [PMID: 20333784]

20 **Malekzadeh R**, Sachdev A, Fahid Ali A. Coeliac disease in developing countries: Middle East, India and North Africa. *Best Pract Res Clin Gastroenterol* 2005; **19**: 351-358 [PMID: 15925841 DOI: 10.1016/j.bpg.2005.01.004]

21 **Catassi C**, Doloretta Macis M, Rätsch IM, De Virgiliis S, Cucca F. The distribution of DQ genes in the Saharawi population provides only a partial explanation for the high celiac disease prevalence. *Tissue Antigens* 2001; **58**: 402-406 [PMID: 11929591]

22 **Catassi C**, Rätsch IM, Gandolfi L, Pratesi R, Fabiani E, El Asmar R, Frijia M, Bearzi I, Vizzoni L. Why is coeliac disease endemic in the people of the Sahara? *Lancet* 1999; **354**: 647-648 [PMID: 10466670]

23 **Trynka G**, Wijmenga C, van Heel DA. A genetic perspective on coeliac disease. *Trends Mol Med* 2010; **16**: 537-550 [PMID: 20947431 DOI: 10.1016/j.molmed.2010.09.003]

24 **Romanos J**, van Diemen CC, Nolte IM, Trynka G, Zhernakova A, Fu J, Bardella MT, Barisani D, McManus R, van Heel DA, Wijmenga C. Analysis of HLA and non-HLA alleles can identify individuals at high risk for celiac disease. *Gastroenterology* 2009; **137**: 834-840, 840.e1-3 [PMID: 19454285 DOI: 10.1053/j.gastro.2009.05.040]

25 **Dieli-Crimi R**, Cénit MC, Núñez C. The genetics of celiac disease: A comprehensive review of clinical implications. *J Autoimmun* 2015; **64**: 26-41 [PMID: 26194613 DOI: 10.1016/j.jaut.2015.07.003]

26 **Sollid LM**, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer. *J Exp Med* 1989; **169**: 345-350 [PMID: 2909659]

27 **Kuja-Halkola R**, Lebwohl B, Halfvarson J, Wijmenga C, Magnusson PK, Ludvigsson JF. Heritability of non-HLA genetics in coeliac disease: a population-based study in 107 000 twins. *Gut* 2016; **65**: 1793-1798 [PMID: 27207974 DOI: 10.1136/gutjnl-2016-311713]

28 **Dubois PC**, Trynka G, Franke L, Hunt KA, Romanos J, Curtotti A, Zhernakova A, Heap GA, Adány R, Aromaa A, Bardella MT, van den Berg LH, Bockett NA, de la Concha EG, Dema B, Fehrmann RS, Fernández-Arquero M, Fiatal S, Grandone E, Green PM, Groen HJ, Gwilliam R, Houwen RH, Hunt SE, Kaukinen K, Kelleher D, Korponay-Szabo I, Kurppa K, MacMathuna P, Mäki M, Mazzilli MC, McCann OT, Mearin ML, Mein CA, Mirza MM, Mistry V, Mora B, Morley KI, Mulder CJ, Murray JA, Núñez C, Oosterom E, Ophoff RA, Polanco I, Peltonen L, Platteel M, Rybak A, Salomaa V, Schweizer JJ, Sperandeo MP, Tack GJ, Turner G, Veldink JH, Verbeek WH, Weersma RK, Wolters VM, Urcelay E, Cukrowska B, Greco L, Neuhausen SL, McManus R, Barisani D, Deloukas P, Barrett JC, Saavalainen P, Wijmenga C, van Heel DA. Multiple common variants for celiac disease influencing immune gene expression. *Nat Genet* 2010; **42**: 295-302 [PMID: 20190752 DOI: 10.1038/ng.543]

29 **Freeman HJ**, Chopra A, Clandinin MT, Thomson AB. Recent advances in celiac disease. *World J Gastroenterol* 2011; **17**: 2259-2272 [PMID: 21633592 DOI: 10.3748/wjg.v17.i18.2259]

30 **Wolters VM**, Verbeek WH, Zhernakova A, Onland-Moret C, Schreurs MW, Monsuur AJ, Verduijn W, Wijmenga C, Mulder CJ. The MYO9B gene is a strong risk factor for developing refractory celiac disease. *Clin Gastroenterol Hepatol* 2007; **5**: 1399-1405, 1405.e1-2 [PMID: 17967566 DOI: 10.1016/j.cgh.2007.08.018]

31 **Qiao SW**, Sollid LM, Blumberg RS. Antigen presentation in celiac disease. *Curr Opin Immunol* 2009; **21**: 111-117 [PMID: 19342211 DOI: 10.1016/j.coi.2009.03.004]

32 **Ráki M**, Schjetne KW, Stamnaes J, Molberg Ø, Jahnsen FL, Issekutz TB, Bogen B, Sollid LM. Surface expression of transglutaminase 2 by dendritic cells and its potential role for uptake and presentation of gluten peptides to T cells. *Scand J Immunol* 2007; **65**: 213-220 [PMID: 17309775 DOI: 10.1111/j.1365-3083.2006.01881.x]

33 **Troncone R**, Auricchio S. Rotavirus and celiac disease: clues to the pathogenesis and perspectives on prevention. *J Pediatr Gastroenterol Nutr* 2007; **44**: 527-528 [PMID: 17460483 DOI: 10.1097/MPG.0b013e31804ca0ec]

34 **Troncone R**, Jabri B. Coeliac disease and gluten sensitivity. *J Intern Med* 2011; **269**: 582-590 [PMID: 21481018 DOI: 10.1111/j.1365-2796.2011.02385.x]

35 **Stene LC**, Honeyman MC, Hoffenberg EJ, Haas JE, Sokol RJ, Emery L, Taki I, Norris JM, Erlich HA, Eisenbarth GS, Rewers M. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. *Am J Gastroenterol* 2006; **101**: 2333-2340 [PMID: 17032199 DOI: 10.1111/j.1572-0241.2006.00741.x]

36 **Pinier M**, Fuhrmann G, Verdu EF, Leroux JC. Prevention measures and exploratory pharmacological treatments of celiac disease. *Am J Gastroenterol* 2010; **105**: 2551-2561; quiz 2562 [PMID: 20877349 DOI: 10.1038/ajg.2010.372]

37 **Catassi C**, Fabiani E, Iacono G, D'Agate C, Francavilla R, Biagi F, Volta U, Accomando S, Picarelli A, De Vitis I, Pianelli G, Gesuita R, Carle F, Mandolesi A, Bearzi I, Fasano A. A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. *Am J Clin Nutr* 2007; **85**: 160-166 [PMID: 17209192]

38 **Silano M**, Agostoni C, Guandalini S. Effect of the timing of gluten introduction on the development of celiac disease. *World J Gastroenterol* 2010; **16**: 1939-1942 [PMID: 20419830]

39 **Sanz Y**, De Pama G, Laparra M. Unraveling the ties between celiac disease and intestinal microbiota. *Int Rev Immunol* 2011; **30**: 207-218 [PMID: 21787226 DOI: 10.3109/08830185.2011.599084]

40 **Elli L**, Rossi V, Conte D, Ronchi A, Tomba C, Passoni M, Bardella MT, Roncoroni L, Guzzi G. Increased Mercury Levels in Patients with Celiac Disease following a Gluten-Free Regimen. *Gastroenterol Res Pract* 2015; **2015**: 953042 [PMID: 25802516 DOI: 10.1155/2015/953042]

41 **Lerner A**, Matthias T. Possible association between celiac disease and bacterial transglutaminase in food processing: a hypothesis. *Nutr Rev* 2015; **73**: 544-552 [PMID: 26084478 DOI: 10.1093/nutrit/nuv011]

42 **du Pré MF**, Sollid LM. T-cell and B-cell immunity in celiac disease. *Best Pract Res Clin Gastroenterol* 2015; **29**: 413-423 [PMID: 26060106 DOI: 10.1016/j.bpg.2015.04.001]

43 **Kim SM**, Mayassi T, Jabri B. Innate immunity: actuating the gears of celiac disease pathogenesis. *Best Pract Res Clin Gastroenterol* 2015; **29**: 425-435 [PMID: 26060107 DOI: 10.1016/j.bpg.2015.05.001]

44 **Mazzarella G**. Effector and suppressor T cells in celiac disease. *World J Gastroenterol* 2015; **21**: 7349-7356 [PMID: 26139981 DOI: 10.3748/wjg.v21.i24.7349]

45 **Björck S**, Lindehammer SR, Fex M, Agardh D. Serum cytokine pattern in young children with screening detected coeliac disease. *Clin Exp Immunol* 2015; **179**: 230-235 [PMID: 25212572 DOI: 10.1111/cei.12454]

46 **Matthias T**, Neidhöfer S, Pfeiffer S, Prager K, Reuter S, Gershwin ME. Novel trends in celiac disease. *Cell Mol Immunol* 2011; **8**: 121-125 [PMID: 21278765 DOI: 10.1038/cmi.2010.68]

47 **Hayat M**, Cairns A, Dixon MF, O'Mahony S. Quantitation of intraepithelial lymphocytes in human duodenum: what is normal? *J Clin Pathol* 2002; **55**: 393-394 [PMID: 11986350]

48 **Veress B**, Franzén L, Bodin L, Borch K. Duodenal intraepithelial lymphocyte-count revisited. *Scand J Gastroenterol* 2004; **39**: 138-144 [PMID: 15000275]

49 **Mubarak A**, Wolters VM, Houwen RH, ten Kate FJ. Immunohistochemical CD3 staining detects additional patients with celiac disease. *World J Gastroenterol* 2015; **21**: 7553-7557 [PMID: 26140002 DOI: 10.3748/wjg.v21.i24.7553]

50 **Hudacko R**, Kathy Zhou X, Yantiss RK. Immunohistochemical stains for CD3 and CD8 do not improve detection of gluten-sensitive enteropathy in duodenal biopsies. *Mod Pathol* 2013; **26**: 1241-1245 [PMID: 23558576 DOI: 10.1038/modpathol.2013.57]

51 **Elli L**, Zini E, Tomba C, Bardella MT, Bosari S, Conte D, Runza L, Roncoroni L, Ferrero S. Histological evaluation of duodenal biopsies from coeliac patients: the need for different grading criteria during follow-up. *BMC Gastroenterol* 2015; **15**: 133 [PMID: 26467310 DOI: 10.1186/s12876-015-0361-8]

52 **Marsh MN**. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). *Gastroenterology* 1992; **102**: 330-354 [PMID: 1727768]

53 **Oberhuber G**. Histopathology of celiac disease. *Biomed Pharmacother* 2000; **54**: 368-372 [PMID: 10989975 DOI: 10.1016/S0753-3322(01)80003-2]

54 **Mills JR**, Murray JA. Contemporary celiac disease diagnosis: is a biopsy avoidable? *Curr Opin Gastroenterol* 2016; **32**: 80-85 [PMID: 26784474 DOI: 10.1097/MOG.0000000000000245]

55 **Oberhuber G**, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur J Gastroenterol Hepatol* 1999; **11**: 1185-1194 [PMID: 10524652]

56 **Ludvigsson JF**, Leffler DA, Bai JC, Biagi F, Fasano A, Green PH, Hadjivassiliou M, Kaukinen K, Kelly CP, Leonard JN, Lundin KE, Murray JA, Sanders DS, Walker MM, Zingone F, Ciacci C. The Oslo definitions for coeliac disease and related terms. *Gut* 2013; **62**: 43-52 [PMID: 22345659 DOI: 10.1136/gutjnl-2011-301346]

57 **Tully MA**. Pediatric celiac disease. *Gastroenterol Nurs* 2008; **31**: 132-140; quiz 141-142 [PMID: 18391801 DOI: 10.1097/01.SGA.0000316532.69484.44]

58 **Troncone R**, Greco L, Mayer M, Paparo F, Caputo N, Micillo M,

Mugione P, Auricchio S. Latent and potential coeliac disease. *Acta Paediatr Suppl* 1996; **412**: 10-14 [PMID: 8783748]
59 **Ho-Yen C**, Chang F, van der Walt J, Mitchell T, Ciclitira P. Recent advances in refractory coeliac disease: a review. *Histopathology* 2009; **54**: 783-795 [PMID: 18700844 DOI: 10.1111/j.1365-2559.2008.03112.x]
60 **Rodrigo-Sáez L**, Fuentes-Álvarez D, Pérez-Martínez I, Alvarez-Mieres N, Niño-García P, de-Francisco-García R, Riestra-Menéndez S, Bousoño-García C, Alonso-Arias R, López-Vázquez A. Differences between pediatric and adult celiac disease. *Rev Esp Enferm Dig* 2011; **103**: 238-244 [PMID: 21619387]
61 **Barker JM**, Liu E. Celiac disease: pathophysiology, clinical manifestations, and associated autoimmune conditions. *Adv Pediatr* 2008; **55**: 349-365 [PMID: 19048738]
62 **Akirov A**, Pinhas-Hamiel O. Co-occurrence of type 1 diabetes mellitus and celiac disease. *World J Diabetes* 2015; **6**: 707-714 [PMID: 26069719 DOI: 10.4239/wjd.v6.i5.707]
63 **Ludvigsson JF**, Ludvigsson J, Ekbom A, Montgomery SM. Celiac disease and risk of subsequent type 1 diabetes: a general population cohort study of children and adolescents. *Diabetes Care* 2006; **29**: 2483-2488 [PMID: 17065689 DOI: 10.2337/dc06-0794]
64 **Bao F**, Yu L, Babu S, Wang T, Hoffenberg EJ, Rewers M, Eisenbarth GS. One third of HLA DQ2 homozygous patients with type 1 diabetes express celiac disease-associated transglutaminase autoantibodies. *J Autoimmun* 1999; **13**: 143-148 [PMID: 10441179 DOI: 10.1006/jaut.1999.0303]
65 **Ch'ng CL**, Jones MK, Kingham JG. Celiac disease and autoimmune thyroid disease. *Clin Med Res* 2007; **5**: 184-192 [PMID: 18056028 DOI: 10.3121/cmr.2007.738]
66 **Norris JM**, Barriga K, Klingensmith G, Hoffman M, Eisenbarth GS, Erlich HA, Rewers M. Timing of initial cereal exposure in infancy and risk of islet autoimmunity. *JAMA* 2003; **290**: 1713-1720 [PMID: 14519705 DOI: 10.1001/jama.290.13.1713]
67 **Norris JM**, Barriga K, Hoffenberg EJ, Taki I, Miao D, Haas JE, Emery LM, Sokol RJ, Erlich HA, Eisenbarth GS, Rewers M. Risk of celiac disease autoimmunity and timing of gluten introduction in the diet of infants at increased risk of disease. *JAMA* 2005; **293**: 2343-2351 [PMID: 15900004 DOI: 10.1001/jama.293.19.2343]
68 **Ziegler AG**, Schmid S, Huber D, Hummel M, Bonifacio E. Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies. *JAMA* 2003; **290**: 1721-1728 [PMID: 14519706 DOI: 10.1001/jama.290.13.1721]
69 **Hummel M**, Bonifacio E, Naserke HE, Ziegler AG. Elimination of dietary gluten does not reduce titers of type 1 diabetes-associated autoantibodies in high-risk subjects. *Diabetes Care* 2002; **25**: 1111-1116 [PMID: 12087006]
70 **Stazi AV**, Trinti B. Selenium status and over-expression of interleukin-15 in celiac disease and autoimmune thyroid diseases. *Ann Ist Super Sanita* 2010; **46**: 389-399 [PMID: 21169670 DOI: 10.4415/ANN_10_04_06]
71 **Anania C**, De Luca E, De Castro G, Chiesa C, Pacifico L. Liver involvement in pediatric celiac disease. *World J Gastroenterol* 2015; **21**: 5813-5822 [PMID: 26019445 DOI: 10.3748/wjg.v21.i19.5813]
72 **Bardella MT**, Vecchi M, Conte D, Del Ninno E, Fraquelli M, Pacchetti S, Minola E, Landoni M, Cesana BM, De Franchis R. Chronic unexplained hypertransaminasemia may be caused by occult celiac disease. *Hepatology* 1999; **29**: 654-657 [PMID: 10051464 DOI: 10.1002/hep.510290318]
73 **Rubio-Tapia A**, Murray JA. The liver in celiac disease. *Hepatology* 2007; **46**: 1650-1658 [PMID: 17969053 DOI: 10.1002/hep.21949]
74 **González-Abraldes J**, Sánchez-Fueyo A, Bessa X, Moitinho E, Feu F, Mas A, Escorsell A, Bruguera M. Persistent hypertransaminasemia as the presenting feature of celiac disease. *Am J Gastroenterol* 1999; **94**: 1095-1097 [PMID: 10201490 DOI: 10.1111/j.1572-0241.1999.01021.x]
75 **Volta U**. Pathogenesis and clinical significance of liver injury in celiac disease. *Clin Rev Allergy Immunol* 2009; **36**: 62-70 [PMID: 18496773 DOI: 10.1007/s12016-008-8086-x]
76 **Zali MR**, Rostami Nejad M, Rostami K, Alavian SM. Liver complications in celiac disease. *Hepat Mon* 2011; **11**: 333-341 [PMID: 22087157]
77 **Bushara KO**. Neurologic presentation of celiac disease. *Gastroenterology* 2005; **128**: S92-S97 [PMID: 15825133]
78 **Grossman G**. Neurological complications of coeliac disease: what is the evidence? *Pract Neurol* 2008; **8**: 77-89 [PMID: 18344378 DOI: 10.1136/jnnp.2007.139717]
79 **Gobbi G**. Coeliac disease, epilepsy and cerebral calcifications. *Brain Dev* 2005; **27**: 189-200 [PMID: 15737700 DOI: 10.1016/j.braindev.2004.05.003]
80 **Smedby KE**, Akerman M, Hildebrand H, Glimelius B, Ekbom A, Askling J. Malignant lymphomas in coeliac disease: evidence of increased risks for lymphoma types other than enteropathy-type T cell lymphoma. *Gut* 2005; **54**: 54-59 [PMID: 15591504 DOI: 10.1136/gut.2003.032094]
81 **Richir M**, Songun I, Wientjes C, Snel P, Dwars B. Small Bowel Adenocarcinoma in a Patient with Coeliac Disease: Case Report and Review of the Literature. *Case Rep Gastroenterol* 2010; **4**: 416-420 [PMID: 21060711 DOI: 10.1159/000313547]
82 **Ozgör B**, Selimoğlu MA. Coeliac disease and reproductive disorders. *Scand J Gastroenterol* 2010; **45**: 395-402 [PMID: 20017709 DOI: 10.3109/00365520903508902]
83 **Volta U**, Villanacci V. Celiac disease: diagnostic criteria in progress. *Cell Mol Immunol* 2011; **8**: 96-102 [PMID: 21278763 DOI: 10.1038/cmi.2010.64]
84 **Ludvigsson JF**, Green PH. Clinical management of coeliac disease. *J Intern Med* 2011; **269**: 560-571 [PMID: 21443532 DOI: 10.1111/j.1365-2796.2011.02379.x]
85 **Rubio-Tapia A**, Hill ID, Kelly CP, Calderwood AH, Murray JA. ACG clinical guidelines: diagnosis and management of celiac disease. *Am J Gastroenterol* 2013; **108**: 656-676; quiz 677 [PMID: 23609613 DOI: 10.1038/ajg.2013.79]
86 **Villanacci V**, Ceppa P, Tavani E, Vindigni C, Volta U. Coeliac disease: the histology report. *Dig Liver Dis* 2011; **43** Suppl 4: S385-S395 [PMID: 21459344 DOI: 10.1016/S1590-8658(11)60594-X]
87 **Lerner A**, Matthias T. Rheumatoid arthritis-celiac disease relationship: joints get that gut feeling. *Autoimmun Rev* 2015; **14**: 1038-1047 [PMID: 26190704 DOI: 10.1016/j.autrev.2015.07.007]
88 **Ciaccio EJ**, Bhagat G, Lewis SK, Green PH. Quantitative image analysis of celiac disease. *World J Gastroenterol* 2015; **21**: 2577-2581 [PMID: 25759524 DOI: 10.3748/wjg.v21.i9.2577]
89 **Catassi C**, Fasano A. Celiac disease diagnosis: simple rules are better than complicated algorithms. *Am J Med* 2010; **123**: 691-693 [PMID: 20670718 DOI: 10.1016/j.amjmed.2010.02.019]
90 **Khalesi M**, Jafari SA, Kiani M, Picarelli A, Borghini R, Sadeghi R, Eghtedar A, Ayatollahi H, Kianifar HR. In Vitro Gluten Challenge Test for Celiac Disease Diagnosis. *J Pediatr Gastroenterol Nutr* 2016; **62**: 276-283 [PMID: 26196202 DOI: 10.1097/MPG.0000000000000917]
91 **Hassan K**, A-Kader H. Celiac disease: the search for adjunctive or alternative therapies. *Expert Rev Gastroenterol Hepatol* 2014; **8**: 313-321 [PMID: 24490653 DOI: 10.1586/17474124.2014.882769]
92 **Makharia GK**. Current and emerging therapy for celiac disease. *Front Med* (Lausanne) 2014; **1**: 6 [PMID: 25705619 DOI: 10.3389/fmed.2014.00006]
93 **Plugis NM**, Khosla C. Therapeutic approaches for celiac disease. *Best Pract Res Clin Gastroenterol* 2015; **29**: 503-521 [PMID: 26060114 DOI: 10.1016/j.bpg.2015.04.005]
94 **Malamut G**, Cellier C. Is refractory celiac disease more severe in old Europe? *Am J Gastroenterol* 2011; **106**: 929-932 [PMID: 21540899 DOI: 10.1038/ajg.2011.105]
95 **Dray X**, Joly F, Lavergne-Slove A, Treton X, Bouhnik Y, Messing B. A severe but reversible refractory sprue. *Gut* 2006; **55**: 1210-1211 [PMID: 16849355 DOI: 10.1136/gut.2005.089987]
96 **Al-Toma A**, Verbeek WH, Mulder CJ. Update on the management of refractory coeliac disease. *J Gastrointestin Liver Dis* 2007; **16**: 57-63 [PMID: 17410290]
97 **Norsa L**, Tomba C, Agostoni C, Branchi F, Bardella MT, Roncoroni L, Conte D, Elli L. Gluten-free diet or alternative therapy: a survey on what parents of celiac children want. *Int J Food Sci Nutr* 2015; **66**: 590-594 [PMID: 26171630 DOI: 10.3109/09637486.2015.1064872]

98 **Tomal J**, McKiernan D, Guandalini S, Semrad CE, Kupfer S. Celiac patients' attitudes regarding novel therapies. *Minerva Gastroenterol Dietol* 2016; **62**: 275-280 [PMID: 27405659]

99 **Fasano A**, Araya M, Bhatnagar S, Cameron D, Catassi C, Dirks M, Mearin ML, Ortigosa L, Phillips A. Federation of International Societies of Pediatric Gastroenterology, Hepatology, and Nutrition consensus report on celiac disease. *J Pediatr Gastroenterol Nutr* 2008; **47**: 214-219 [PMID: 18664878 DOI: 10.1097/MPG.0b013e3-18181afed]

100 **Siegel M**, Garber ME, Spencer AG, Botwick W, Kumar P, Williams RN, Kozuka K, Shreeniwas R, Pratha V, Adelman DC. Safety, tolerability, and activity of ALV003: results from two phase 1 single, escalating-dose clinical trials. *Dig Dis Sci* 2012; **57**: 440-450 [PMID: 21948339 DOI: 10.1007/s10620-011-1906-5]

101 **Tye-Din JA**, Anderson RP, Ffrench RA, Brown GJ, Hodsman P, Siegel M, Botwick W, Shreeniwas R. The effects of ALV003 pre-digestion of gluten on immune response and symptoms in celiac disease in vivo. *Clin Immunol* 2010; **134**: 289-295 [PMID: 19942485 DOI: 10.1016/j.clim.2009.11.001]

102 **Mitea C**, Havenaar R, Drijfhout JW, Edens L, Dekking L, Koning F. Efficient degradation of gluten by a prolyl endoprotease in a gastrointestinal model: implications for coeliac disease. *Gut* 2008; **57**: 25-32 [PMID: 17494108 DOI: 10.1136/gut.2006.111609]

103 **Setty M**, Hormaza L, Guandalini S. Celiac disease: risk assessment, diagnosis, and monitoring. *Mol Diagn Ther* 2008; **12**: 289-298 [PMID: 18803427]

104 **Sollid LM**, Khosla C. Novel therapies for coeliac disease. *J Intern Med* 2011; **269**: 604-613 [PMID: 21401739 DOI: 10.1111/j.1365-2796.2011.02376.x]

105 **Schuppan D**, Junker Y, Barisani D. Celiac disease: from pathogenesis to novel therapies. *Gastroenterology* 2009; **137**: 1912-1933 [PMID: 19766641 DOI: 10.1053/j.gastro.2009.09.008]

106 **Utech M**, Ivanov AI, Samarin SN, Bruewer M, Turner JR, Mrsny RJ, Parkos CA, Nusrat A. Mechanism of IFN-gamma-induced endocytosis of tight junction proteins: myosin II-dependent vacuolarization of the apical plasma membrane. *Mol Biol Cell* 2005; **16**: 5040-5052 [PMID: 16055505 DOI: 10.1091/mbc.E05-03-0193]

107 **McKerracher L**, Higuchi H. Targeting Rho to stimulate repair after spinal cord injury. *J Neurotrauma* 2006; **23**: 309-317 [PMID: 16629618 DOI: 10.1089/neu.2006.23.309]

108 **González-Mariscal L**, Lechuga S, Garay E. Role of tight junctions in cell proliferation and cancer. *Prog Histochem Cytochem* 2007; **42**: 1-57 [PMID: 17502225 DOI: 10.1016/j.proghi.2007.01.001]

109 **Bargetzi MJ**, Schönenberger A, Tichelli A, Fried R, Cathomas G, Signer E, Speck B, Gratwohl A. Celiac disease transmitted by allogeneic non-T cell-depleted bone marrow transplantation. *Bone Marrow Transplant* 1997; **20**: 607-609 [PMID: 9337064 DOI: 10.1038/sj.bmt.1700926]

110 **Rashtak S**, Murray JA. Review article: coeliac disease, new approaches to therapy. *Aliment Pharmacol Ther* 2012; **35**: 768-781 [PMID: 22324389 DOI: 10.1111/j.1365-2036.2012.05013.x]

111 **Salvati VM**, Mazzarella G, Gianfrani C, Levings MK, Stefanile R, De Giulio B, Iaquinto G, Giardullo N, Auricchio S, Roncarolo MG, Troncone R. Recombinant human interleukin 10 suppresses gliadin dependent T cell activation in ex vivo cultured coeliac intestinal mucosa. *Gut* 2005; **54**: 46-53 [PMID: 15591503 DOI: 10.1136/gut.2003.023150]

112 **Gillett HR**, Arnott ID, McIntyre M, Campbell S, Dahele A, Priest M, Jackson R, Ghosh S. Successful infliximab treatment for steroid-refractory celiac disease: a case report. *Gastroenterology* 2002; **122**: 800-805 [PMID: 11875014]

113 **Costantino G**, della Torre A, Lo Presti MA, Caruso R, Mazzon E, Fries W. Treatment of life-threatening type I refractory coeliac disease with long-term infliximab. *Dig Liver Dis* 2008; **40**: 74-77 [PMID: 17382609 DOI: 10.1016/j.dld.2006.10.017]

114 **Ghosh S**, Goldin E, Gordon FH, Malchow HA, Rask-Madsen J, Rutgeerts P, Vyhnálek P, Zádorová Z, Palmer T, Donoghue S. Natalizumab for active Crohn's disease. *N Engl J Med* 2003; **348**: 24-32 [PMID: 12510039 DOI: 10.1056/NEJMoa020732]

115 **Crespo Pérez L**, Castillejo de Villasante G, Cano Ruiz A, León F. Non-dietary therapeutic clinical trials in coeliac disease. *Eur J Intern Med* 2012; **23**: 9-14 [PMID: 22153524 DOI: 10.1016/j.ejim.2011.08.030]

116 **Hopman EG**, von Blomberg ME, Batstra MR, Morreau H, Dekker FW, Koning F, Lamers CB, Mearin ML. Gluten tolerance in adult patients with celiac disease 20 years after diagnosis? *Eur J Gastroenterol Hepatol* 2008; **20**: 423-429 [PMID: 18403944 DOI: 10.1097/MEG.0b013e3282f4de6e]

117 **Bardella MT**, Fredella C, Trovato C, Ermacora E, Cavalli R, Saladino V, Prampolini L. Long-term remission in patients with dermatitis herpetiformis on a normal diet. *Br J Dermatol* 2003; **149**: 968-971 [PMID: 14632800]

118 **Huibregtse IL**, Marietta EV, Rashtak S, Koning F, Rottiers P, David CS, van Deventer SJ, Murray JA. Induction of antigen-specific tolerance by oral administration of Lactococcus lactis delivered immunodominant DQ8-restricted gliadin peptide in sensitized nonobese diabetic Abo Dq8 transgenic mice. *J Immunol* 2009; **183**: 2390-2396 [PMID: 19635921 DOI: 10.4049/jimmunol.0802891]

119 **Daveson AJ**, Jones DM, Gaze S, McSorley H, Clouston A, Pascoe A, Cooke S, Speare R, Macdonald GA, Anderson R, McCarthy JS, Loukas A, Croese J. Effect of hookworm infection on wheat challenge in celiac disease--a randomised double-blinded placebo controlled trial. *PLoS One* 2011; **6**: e17366 [PMID: 21408161 DOI: 10.1371/journal.pone.0017366]

120 **Scanlon SA**, Murray JA. Update on celiac disease - etiology, differential diagnosis, drug targets, and management advances. *Clin Exp Gastroenterol* 2011; **4**: 297-311 [PMID: 22235174 DOI: 10.2147/CEG.S8315]


**P- Reviewer**: Krupa-Kozak U, Vasudevan A **S- Editor**: Song XX
**L- Editor**: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pathophysiology

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

*World J Gastrointest Pathophysiol* 2017 May 15; 8(2): 39-50

DOI: 10.4291/wjgp.v8.i2.39

ISSN 2150-5330 (online)

REVIEW

# Embrionary way to create a fatty liver in portal hypertension

Maria-Angeles Aller, Natalia Arias, Isabel Peral, Sara García-Higarza, Jorge-Luis Arias, Jaime Arias

Maria-Angeles Aller, Isabel Peral, Jaime Arias, Department of Surgery, School of Medicine, Complutense University of Madrid, 28040 Madrid, Spain

Natalia Arias, UCL Division of Medicine, Institute for Liver and Digestive Health, London NW3 2PF, United Kingdom

Sara García-Higarza, Jorge-Luis Arias, Laboratory of Neuroscience, Department of Psychology, University of Oviedo, 33003 Asturias, Spain

Author contributions: Aller MA, Arias N and Arias J wrote the manuscript; Peral I, García-Higarza S and Arias JL contributed to the manuscript by providing intellectual input, and have participated in preparing the manuscript and approved its final version.

Supported by FICYT FC-15-GRUPIN 14-088 and Alfonso Martin Escudero Foundation.

Conflict-of-interest statement: The authors declare that there are any conflict of interest.



Manuscript source: Invited manuscript

Correspondence to: Maria-Angeles Aller, MD, PhD, Department of Surgery, School of Medicine, Complutense University of Madrid, Plaza de Ramón y Cajal s.n., 28040 Madrid, Spain. maaller@med.ucm.es
Telephone: +34-91-3941388
Fax: +34-91-3947115

Received: December 5, 2016
Peer-review started: December 7, 2016
First decision: January 16, 2017
Revised: February 3, 2017
Accepted: February 28, 2017

Article in press: March 2, 2017
Published online: May 15, 2017

## Abstract

Portal hypertension in the rat by triple partial portal vein ligation produces an array of splanchnic and systemic disorders, including hepatic steatosis. In the current review these alterations are considered components of a systemic inflammatory response that would develop through three overlapping phenotypes: The neurogenic, the immune and the endocrine. These three inflammatory phenotypes could resemble the functions expressed during embryonic development of mammals. In turn, the inflammatory phenotypes would be represented in the embryo by two functional axes, that is, a coelomic-amniotic axis and a trophoblastic yolk-sac or vitelline axis. In this sense, the inflammatory response developed after triple partial portal vein ligation in the rat would integrate both functional embryonic axes on the liver interstitial space of Disse. If so, this fact would favor the successive development of steatosis, steatohepatitis and fibrosis. Firstly, these recapitulated embryonic functions would produce the evolution of liver steatosis. In this way, this fat liver could represent a yolk-sac-like in portal hypertensive rats. After that, the systemic recapitulation of these embryonic functions in experimental prehepatic portal hypertension would consequently induce a gastrulation-like response in which a hepatic wound healing reaction or fibrosis occur. In conclusion, studying the mechanisms involved in embryonic development could provide key results for a better understanding of the nonalcoholic fatty liver disease etiopathogeny.

**Key words:** Inflammation; Non-alcoholic fatty liver disease; Hepatic steatosis; Extraembryonic functions; Fibrosis; Portal hypertension

**Core tip:** The current hypothesis proposes that the re-expression of two embryonic systemic functional axes in the rat after partial portal vein ligation produces a non-alcoholic fatty liver disease. These axes, a coelomicamniotic axis and a trophoblastic yolk-sac or vitelline axis, would then integrate in the interstitial liver space of Disse. If so, these recapitulated embryonic functions would produce firstly, the evolution of liver steatosis. In this way, this fat liver could represent a yolk-saclike in portal hypertensive rats. After that, these embryonic functions would induce a gastrulation-like response in which liver fibrosis occcur. For that reason, studying the mechanisms involved in embryonic development could provide key results for a better understanding of the non-alcoholic fatty liver disease pathophysiology.

Aller MA, Arias N, Peral I, García-Higarza S, Arias JL, Arias J. Embrionary way to create a fatty liver in portal hypertension. *World J Gastrointest Pathophysiol* 2017; 8(2): 39-50 Available from: URL: http://www.wjgnet.com/2150-5330/full/v8/i2/39.htm DOI: http://dx.doi.org/10.4291/wjgp.v8.i2.39

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a pathological condition derived from a wide spectrum of liver damage[1-3]. NAFLD covers from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH). While NAFL is characterized by hepatic steatosis without hepatocellular injury such as ballooning of the hepatocytes, NASH condition shows hepatic steatosis, inflammatory state accompanied with hepatocyte injury and ballooning, with or without fibrosis[4]. Although NAFLD is strongly associate with obesity, diabetes mellitus and dyslipidemia, its pathogenesis remains poorly understood and therapeutic options are limited[3,4].

The concept that this range of related hepatic disorders is an evolutive inflammatory condition could simplify the integration of the different etiopathogenic mechanisms involved in each one of its evolutive phases. Even, NAFLD has been already considered like the hepatic manifestation of a systemic auto-inflammatory disorder[5]. The earliest inflammatory phase of NAFLD consists in hepatic steatosis, which is characterized by the deposition of triglycerides inside of hepatocytes[3]. This early evolutive period of the disease is usually clinically silent, which is why its clinical diagnosis is delayed and why it is difficult to identify the risk contribution of the splanchnic and systemic etiopathogenic factors[2].

Using experimental liver steatosis models can be very useful to prevent this gap in etiopathogenic knowledge during the early phase of this disease[6,7]. Therefore, the partial portal vein ligation of the rat can be considered one of the experimental surgical models of hepatic steatosis[8]. We have shown an increase in tryglicerides, diacylglicerol and cholesterol in the liver of these animals, together with a microvesicular hepatocytic fatty infiltration. Moreover, the presence of megamitochondria at short (1 mo) and long-term (1 year)[8-10] has been found. Liver steatosis was described in rats with portal hypertension by Izzet *et al*[11] in 2005, a pathological association suggested in humans 35 years ago[12]. Moreover, the liver parenchyma in portal hypertensive rats, due to the hepatocytic fatty infiltration, is progressively converted into fat[8,10] (Figure 1).

Portal hypertension is a type of vascular pathology due to the pressure of mechanical energy on splanchnic venous circulation[13]. In mammals there are five cellular pathways able to translate mechanical forces into biologic programs, such as integrin-matrix interactions, cytoskeletal strain responses, stretch ion channels, cell traction forces and G-protein-coupled receptors[14,15]. Therefore, mechanical energy may act after TPVL in the rat on the vascular splanchnic endothelium as a stressor stimulus triggered by mechanotransduction[16].

Tissue injury caused by this process can produce a systemic inflammatory response in the body. This response is developed by three successive and overlapping phenotypes: The neurogenic, the immune and the endocrine[17,18]. Similar overlapping could also be expressed in the body through the action of the intrinsic endogenous mechanical energy on the splanchnic venous system after the partial PVL[19]. Therefore during its evolution, prehepatic portal hypertension would induce a low-grade inflammatory response which could be developed during the above mentioned phenotypes, the neurogenic, the immune and the endocrine[19] (Table 1).

## THE NEUROGENIC INFLAMMATORY PHENOTYPE

The systemic inflammatory response in prehepatic portal hypertension could begin with an immediate neuromuscular response including the splanchnic and systemic vascular smooth muscle with vasoconstriction and vasodilation producing an ischemia-reperfusion phenomenon. This pathologic vasomotor response induces local and systemic hemodynamic impairments, *i.e.*, hyperdynamic splanchnic and systemic circulation[20]. Within systemic and splanchnic alterations, hyperdynamic circulation is pointed out in relation to prehepatic portal hypertension[21]. Regarding this, the hyperdynamic or progressive vasodilator syndromes could be triggered by splanchnic and systemic vasodilation. Moreover, multiorgan failure in chronic liver disease could be attributable to this syndrome[21,22]. Once developed, the hyperdynamic syndrome induces tissue hypoxia, stress (oxidative and nitrosative) accompanied by mild edema, and inflammation in tissues and organs causing their dysfunction[19]. Multiple studies

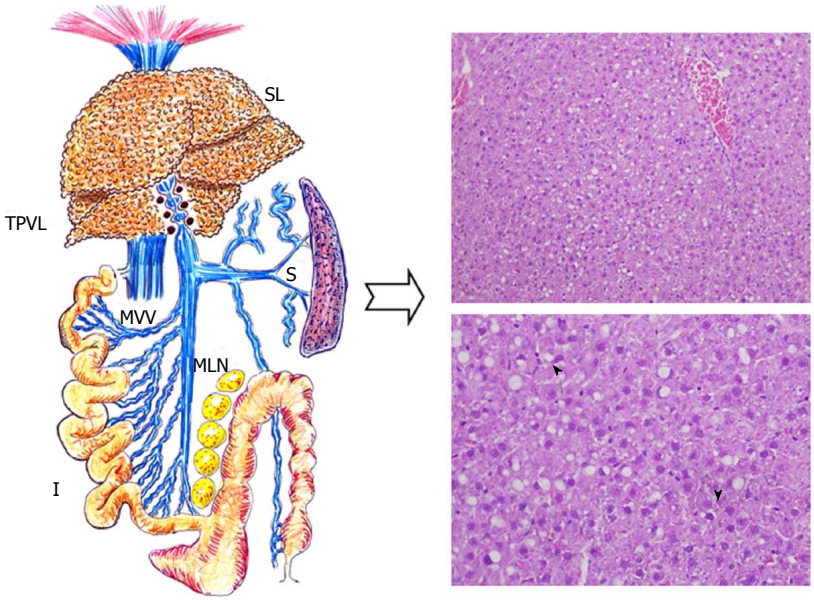


**Figure 1 Liver histopathological changes of a rat after three months with triple partial portal vein ligation.** Hepatic steatotic areas are mainly distributed in zone 1 of the liver acinus, but tipically hepatocyte ballooning is more apparent near zone 3. Both macrovesicular and microvesicular steatosis are evident as well as scattered necroinflammatory foci (arrowheads) (H and E stain, × 200). I: Intestine; MVV: Mesenteric venous vasculopathy; S: Spleen; SL: Steatotic liver; TPVL: Triple partial portal vein ligation.

**Table 1 Similarities between the inflammatory changes developed after triple partial portal vein ligation in the rat and the extra-embryonic functions in mammals**

| Inflammatory phenotype | | Extra-embryonic and embryonic functions |
|---|---|---|
| Neurogenic | Portal ischemia | Coelomic-amniotic functions |
| | Hepatic arterial reperfusion | Neurogenic potential |
| | Neuroendocrine response | Interstitial edema |
| | Hyperdynamic circulation | Bacteriostasis |
| | Mild edema of space of Disse | Anti-inflammation |
| Immune | Kupffer cell and hepatic stellate cell activation | Trophoblastic-yolk sac functions |
| | Leukocyte infiltration | Digestive (phagocytic) functions |
| | Acute phase response | Acute phase proteins |
| | Angiogenesis | Angiogenic switch |
| | Hepatic steatosis | Lipid and protein nutrients (vitellum) |
| Endocrine | Sinusoidal remodeling | Gastrulation-like functions |
| | Capillarization of hepatic sinusoids | Epithelial-mesenchymal transition |
| | Perisinusoidal fibrosis | Intra-embryonic mesenchyma |
| | Steatohepatitis | |

have shown that the central nervous system plays a key role in the pathophysiology of the hyperdynamic circulation[23,24]. In particular c-Fos is detected in the brain stem and hypothalamic nuclei of rats following portal vein ligation[23]. Then, hyperdynamic circulation leads to decreased mean arterial pressure thus stimulating the sympathetic nervous system and the multiple neuro-endocrine axes, including the renin-angiotensin-aldosterone system. This results in sodium retention and volume expansion[20,25,26]. Sodium retention seems to be critical for inflammatory response. Under inflammatory circumstances, endothelium tends to increase permeability in tissues and organs affected. This strategy will allow a selective diffusion of circulating substances from the blood into the interstitial space[27].

Stress of the neuro-endocrine system by portal hypertension could be induce by three mechanisms: The hypothalamic-pituitary-adrenal axis, the sympathetic-adrenal medullary and the sympathetic nervous system[28] (Figure 2). Moreover, the localization of the early inflammatory response could also favor the posterior storage of substances derived from the acute phase response[29]. Therefore, in the liver of the rats with prehepatic portal hypertension, the space of Disse could represent the anatomically definable compartment where the early inflammatory response is expressed (Figure 2).

The main and immediate effect on the liver vascular flow after the partial portal vein ligation is greatly reduced portal blood flow, which normally represents the 70% of its blood supply[30]. Nevertheless, initial portal ischemia is followed by arterial reperfusion. It has been described that decreased portal venous flow is able to induce an increase in hepatic arterial blood flow or "hepatic arterial buffer response"[31]. In turn, this vascular compensatory mechanism arterializes the liver and produces a capillarization of hepatic sinusoids[30]. This phenomenon is defined by fenestrae loss and the formation of a continuous basement membrane[32]. Although the mechanism of defenestration induced in the arterialized liver is not well known, it has been demonstrated that liver sinusoidal endothelial cell phenotype in capillarized

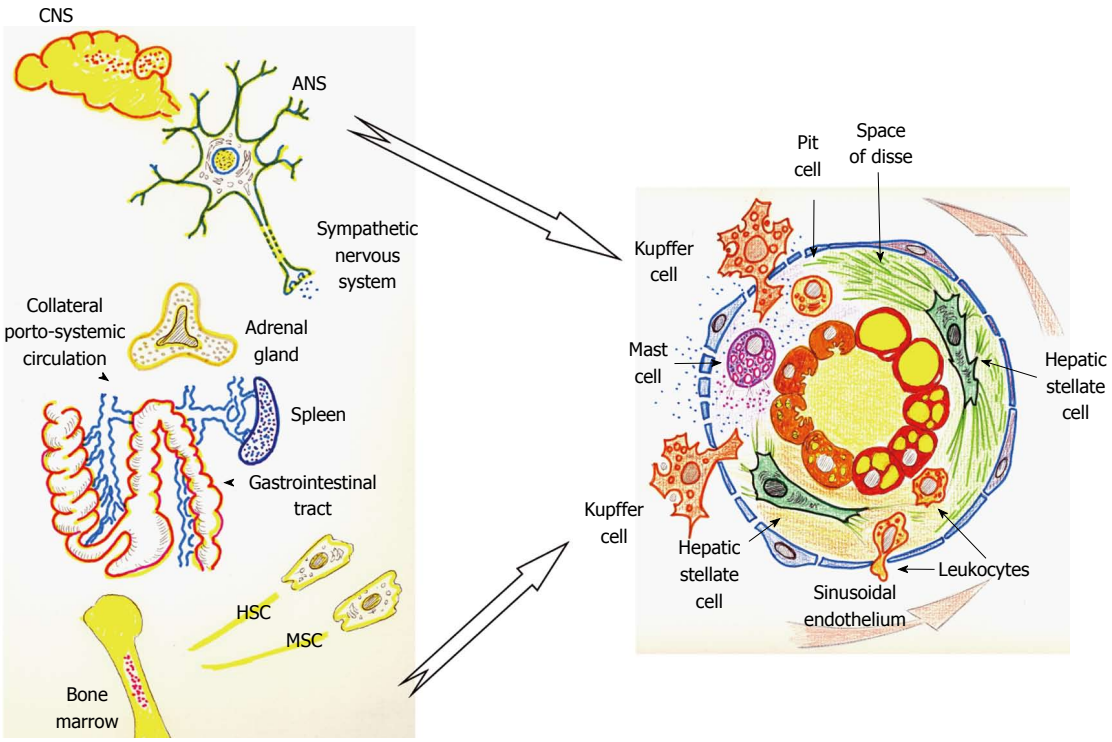


**Figure 2 Inflammatory phenotypes, neurogenic and immune in prehepatic portal hypertensive rat.** ANS: Autonomic nervous system; CNS: Central nervous system; HSC: Hematological stem cell; MSC: Mesenchymal stem cell.

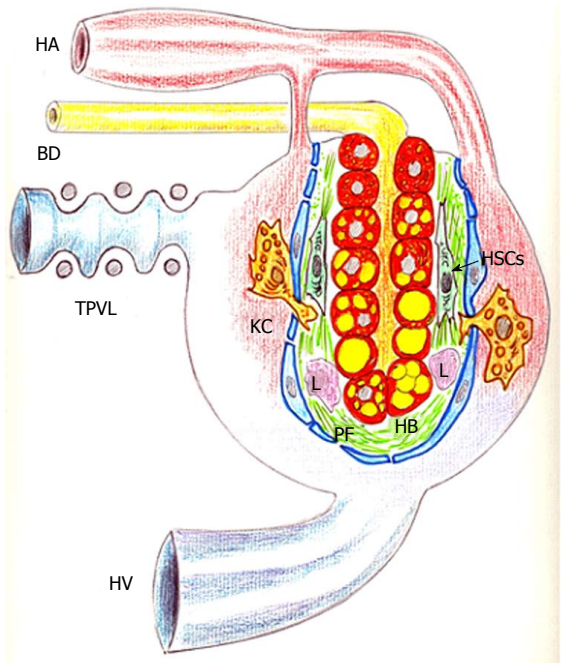


**Figure 3 Schematic representation of the liver parenchyma after partial portal vein in the rat.** The deposit of lipids within the hepatocytes and the liver arterialization, associated with defenestration of the sinusoidal endothelium, and the perisinuosidal fibrosis stand out. BD: Bile duct; HA: Hepatic artery; HV: Hepatic vein; HB: Hepatocyte ballooning; HSCs: Hepatic stellate cells; KC: Kupffer cell; L: Leukocyte; PF: Perisinuosidal fibrosis; TPVL: Triple partial portal vein ligation.

liver promotes Kupffer cell[30] and hepatic stellate cell[33] activation (Figure 3). Moreover, it has been suggested that intrasinusoidal crawling behavior could represent a form of immune surveillance. So, this mechanism could explain the reduction in CD8 T cell surveillance by infected or transformed hepatocytes. Furthermore, intrasinusoidal crawling behavior could participate in the hepatocarcinoma's development and progression[34]. This liver ischemia-reperfusion phenotype with progressing interstitial edema in the space of Disse could activate lymphatic circulation[35]. As a consequence, increased hepatic lymph flow and the transmigration of free cells, such as dendritic cells and lymphocytes, have been shown[34]. In addition, the edematous space of Disse may also serve as a stem cell niche for substances derived from the neuro-endocrine response to portal hypertensive stress, for mediators of the acute phase response and cytokines, and for activated hepatic stellate cells and lymphocytes[35-37] (Figure 4).

## THE IMMUNE INFLAMMATORY PHENOTYPE

The immune inflammatory phenotype by the splanchnic system after ischemia/reperfusion, and therefore suffer oxidative and nitrosative stress, is coupled with the activation of intracellular signaling pathways including the nuclear factor-κB (NF-κB) pathway, and the inflammasome[38]. Reactive oxygen species are central in the regulation of NF-κB, although their role is not completely understood on account of the contrasted outcome reported: Activation *vs* inhibition. Most likely,

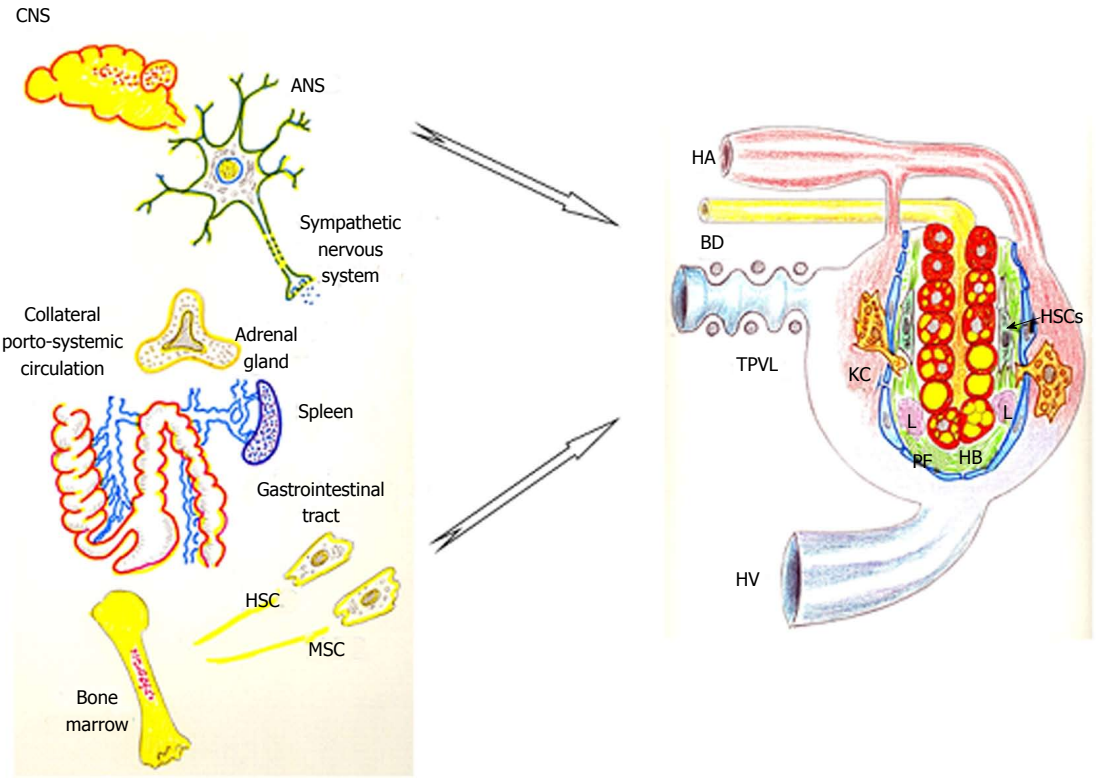


**Figure 4 The preferential expression of the neurogenic and immune inflammatory phenotypes into the space of Disse induces hepatic steatosis, steatohepatitis and fibrosis in portal hypertensive rats by triple partial portal vein ligation.** ANS: Autonomic nervous system; BD: Bile duct; CNS: Central nervous system; HA: Hepatic artery; HB: Hepatocyte ballooning; HSC: Hematological stem cell; HSCs: Hepatic stellate cell; HV: Hepatic vein; KC: Kupffer cell; L: Leukocyte; MSC: Mesenchymal stem cell; PF: Perisinuosidal fibrosis; TPVL: Triple partial portal vein ligation.

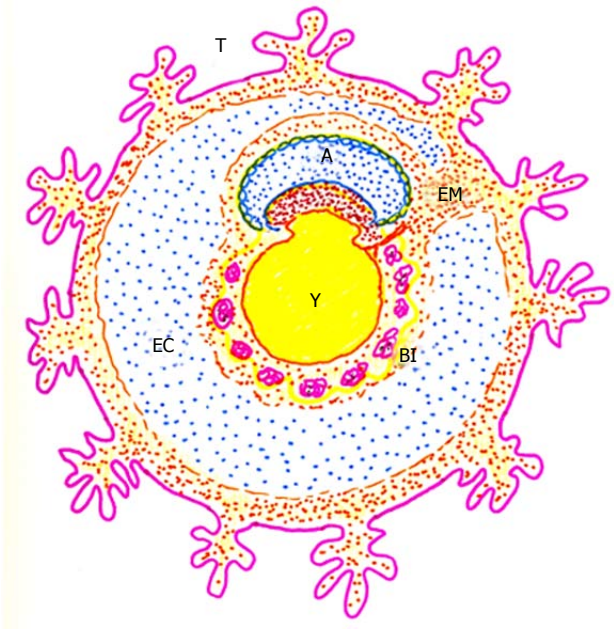


**Figure 5 Schematic representation of early embryo development.** The extra-embryonic mesoderm and the exocoelomic cavity connect the trophoblast with the amnion and the yolk sac. A: Amnion; BI: Blood islands; EC: Exocoelomic cavity; EM: Extra-embryonic mesoderm; T: Trophoblast; Y: Yolk sac.

reactive oxygen species could promote NF-κB responses in the early stages of the inflammatory response instead of inhibiting these responses at later stages. Reactive oxygen and nitrogen species could also favor the induction of tissue repair or remodeling[38-40].

During this phase the oxygen is creating enzymatic stress[19]. Along this acute phase the compensatory mechanisms include production of proteins which bind proteolytic enzymes, inhibitors of leukocyte and lysosomal proteolytic enzymes[41,42]. Likewise, anti-enzymatic stress could be promoted by the natural inhibitors of matrix metalloproteinases[19]. The immune phenotype could be coupled with bacterial intestinal translocation to mesenteric lymph nodes, increased mast cells in the splanchnic area, an acute phase response, dyslipidemia and hepatic steatosis[43]. We have shown that splanchnic and systemic inflammatory changes develop in TPVL-rats, including portal hypertensive enteropathy[43,44], mesenteric adenitis[45,46], portal hypertensive encephalopathy[47], liver steatosis[8-10], aortic atherosclerosis-like disease[48-50] and metabolic syndrome[51].

Hepatic steatosis and visceral adipose tissue are metabolic risk factors in accumulation of visceral fat. Due to their anatomical position, the venous blood from there is drained directly into the liver through the portal vein[52]. We speculate that the induction of intraabdominal fat deposits around the portal venous system could represent ontogenic reminiscences, associated with yolk sac, or phylogenetic reminiscences, related to vitellogenesis[53,54] (Figure 5). Regarding the ontogenic origin, the liver, and in particular the omentum, could

be mimicking the yolk sac, in which pathological lipid deposition takes place. Under this scenario, the liver and the omentum would be regressing to evolutive phases with suitable metabolic conditions, supported by the expression of inflammatory markers such as tumor necrosis factor (TNF)-α, IL-6, C reactive protein and leptin[19,29]. In terms of phylogenetic approach, the body would be adopting the molecular mechanisms related to the vitellogenesis[53,54], in which oviparous species provide a glycolipoprotein yolk storage called vitelline to the egg as food-source for the embryo[54]. Lipoprotein transport through the circulatory system by eukaryotes has been an important function for the existence[53]. Thus, the evolutionary perfection of energy accumulation in fat has provided organisms an advantage in adapting to environmental and developmental changes[54].

The increased uptake of free fatty acids derived from the hydrolysis of adipose-tissue triglycerides results in hepatic steatosis. Moreover, the contribution of dietary chylomicrons, hepatic biogenesis and insulin resistance increased the uptake[3,4,55]. Hepatic steatosis is a key concept in the two-hit NAFLD hypothesis, which postulates that hepatic steatosis sensitizes fatty liver to secondary hits, such as oxidative and nitrosative stress or inflammatory cytokines[56]. In addition, cholesterol sensitizes fatty livers to secondary hits, particularly when trafficking to mitochondria as it has been recently recognized[57,58]. In portal hypertensive rats by TPVL the distinction between simple steatosis and steatohepatitis (NASH) includes hepatocyte ballooning, leukocyte infiltration (lobular inflammation) and perisinusoidal (zone 3) fibrosis[9] (Figures 3 and 4). In long-term portal hypertensive rats, the plasmatic increase of low density lipoprotein and lipopolysaccharide binding protein as well as high-density lipoprotein reduction has been associated with NASH[8-10]. These findings could suggest a NASH role in developing cardiovascular disease by two ways: Systemic release of inflammatory mediators and/or the production of insulin resistance and atherogenic dyslipidemia[49,50]. In this line, recent evidence supports a relationship between NAFLD and cardiovascular disease[50,55]. Particularly in TPVL-rats NASH, this association could be considered a risk factor for a wound-like inflammatory aortic response[49], with increased expression of NF-κB, TNF-α, IL-1β and IL-6 in the aortic wall[48,49].

## THE ENDOCRINE INFLAMMATORY PHENOTYPE

During the expression of the endocrine inflammatory phenotype in TPVL-rats there is a splanchnic remodeling by angiogenesis, which implies growth of new vessels from pre-existing ones[59], and fibrosis. Moreover, an abnormal splanchnic and systemic angioarchitecture, such as portosystemic collaterals have been observed in chronic liver diseases[60-62]. This vascular alteration found in the gastrointestinal tract under portal hypertension conditions is called "hypertensive portal intestinal vasculopathy"[63,64]. However, not only vascular alterations have been described, even histological changes have been found[64]. So, angiogenesis is key role in portal hypertension and represents a potential therapeutic target[65]. Splanchnic hyperemia which is followed by increased splanchnic vascularization, together with portal-systemic collateral circulation in experimental portal hypertension have angiogenic origins partly driven by the vascular endothelial growth factor (VEGF)[65,66]. Mast cells are able to release VEGF among others[67], being involved in promoting hypertensive portal vasculopathy and portal systemic collateral circulation[43].

Fibrosis is a scarring response that seems to be reversible in rats with long-term TPVL. Hepatic stellate cells (HSCs) play a central role in liver fibrosis and homeostasis[68]. In particular, perisinusoidal/pericellular fibrosis is typically found in NASH. Excess deposits of the extracellular matrix are primarily found in the spaces of Disse surrounding sinusoids or groups of hepatocytes. This leads to "capillarization of sinusoids" or "chickenwire pattern", respectively[69] (Figures 3 and 4). HSCs express both mesenchymal markers and neuronal or glial markers[70]. The activation of HSCs is needed to develop hepatic fibrogenesis[68,69]. HSCs activate resident immune cells such as Kupffer and Pitt cells which trigger hepatic inflammation[71], as well as mono- and polymorphonuclear leukocytes infiltration[70].

In addition, HSCs, once in their myofibroblastic phenotype, respond to vasoactive substances by contracting being important in the pathogenesis of portal hypertension[72]. They also respond to sinusoidal morphogenesis since direct hepatic stellate cell-endothelial cell contact inhibits endothelial cell capillary/sinusoidal formation[73]. Dynamic interplay between HSCs and endothelial cells could be important for understanding how the sinusoidal remodeling process is regulated in NAFLD in portal hypertensive rats[73] (Figure 3).

## RECAPITULATED EXTRA-EMBRYONIC FUNCTIONS RELATED TO NAFLD

The inflammatory response associated with NAFLD in portal hypertensive rats could be an ontogenic process based on the re-expression of two speculative extra-embryonic axes (exocoelomic-amniotic and trophoblastic-yolk sac) in the space of Disse. If so, the NAFLD concept which vary from steatosis and NASH to advanced fibrosis and cirrhosis[58] could be representing a normal embryonic development (Table 1).

The hypothetical recapitulation of these initial embryonic phases during the evolution of NAFLD would be accompanied by functions similar to the extra-embryonic membranes that surround the embryo. The extra-embryonic coelom or exocoelomic cavity surrounds the blastocyst, which is composed of two structures, the amnion and the primary yolk sac (Figure 5). Coelomic

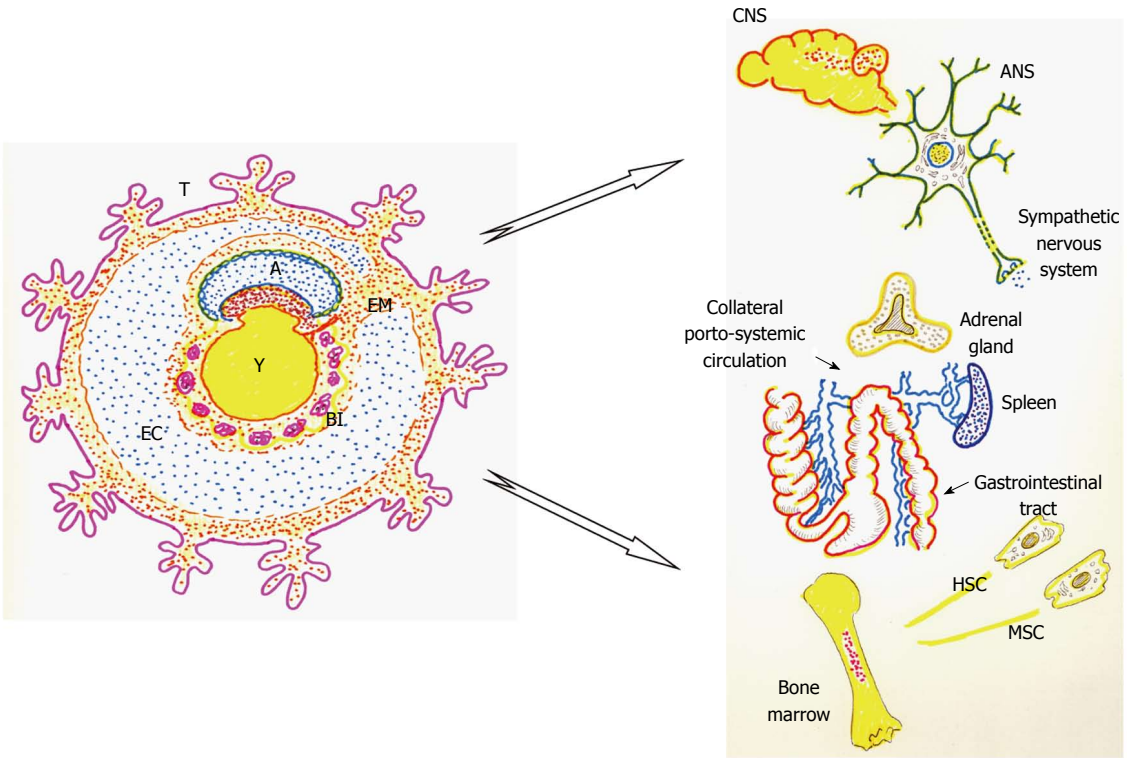


**Figure 6 The coelomic-amniotic functions could be hypothetically recapitulated by the systemic neurogenic phenotype activation, while the trophoblastic-yolk sac functions could be carried out by the systemic immune inflammatory phenotype activation.** A: Amnion; ANS: Autonomic nervous system; BI: Blood islands; CNS: Central nervous system; EC: Exocoelomic cavity; EM: Extra-embryonic mesoderm; HSC: Hematological stem cell; MSC: Mesenchymal stem cell; T: Trophoblast; Y: Yolk sac.

fluid results from an ultrafiltrate of maternal serum with the addition of specific placental and secondary yolk sac byproducts[74]. Accordingly, the embryonic phenotype could be adopted by the inflamed liver interstitium, which will induce fluid accumulation with low pH and oxygen environment as coelomic fluid[74,75]. This interstitial edema seems to occur secondary to hepatic ischemia-reperfusion. Moreover, the edema shows proinflammatory characteristics due to the high content in proteins such as albumin, electrolytes, metals, amino acids, antioxidants, cytokines and cholesterol-derived hormones[75,76]. So the edema will be leading liver trophism.

Biological and anatomical data of the exocoelomic cavity stand it out as a nutritional pathway before placental circulation is established[76]. The strong neural potential of the amnion, an embryonic functional axis, has been previously stated[77]. Moreover, from the amnion is secreted the "amnio-derived cellular cytokine solution" which highlighting a connection between mesenchymal and epithelial cells during embryo development[78]. Furthermore, the amniotic fluid could be understand as an extension of the extracellular space of the fetal tissues[79]. Finally, pluripotent stem cells within the amniotic fluid could also be a new source for stem cell research[80,81]. Because of these characteristics, the amnio-like phenotype could favor nutrition by diffusion, transport, excretion, and bacteriostatic and antiinflammatory protection[78,79] (Figure 5).

The development of hematopoiesis and angiogenesis[81] occurs in the mesenchymal layer which builds the wall of the secondary yolk sac in mammals[74]. This sac appears from the sixth week of gestation and is covered by superficial small vessels[82]. Others layers of the secondary yolk sac include, mesothelial and endodermal layers which are active in endocytosis/digestion and absorptive functions[81,82], and the endodermal layer which produces acute phase proteins, such as transferrine, α1-antitrypsin, and α-fetoprotein (produced by both the adult and fetal liver)[74,83] (Figure 5). So, the yolk sac provides lipids, carbohydrates, proteins and vascular integrity to the embryos[84] and could be involved in lipid metabolism gene regulation[85], immune cells recruitment and angiogenic switch[86]. Phagocytosis has been associated to trophoblast differentiation as well[87].

## THE RECAPITULATED SYSTEMIC EXTRA-EMBRYONIC FUNCTION AND THE INTERSTITIAL INFLAMMATORY SPACE OF DISSE IN NAFLD

NAFLD systemic inflammatory pathophysiology shares mechanisms with the pluripotential extra-embryonic pathways. It is hypothesized that during NAFLD evolu-

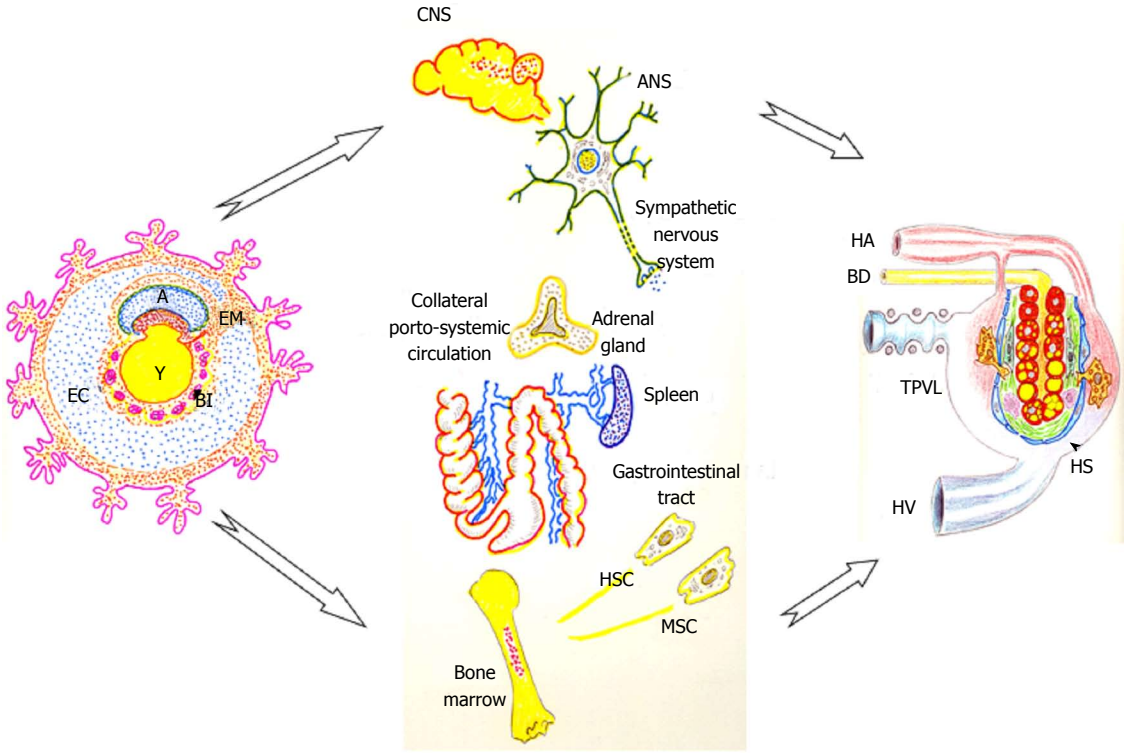


**Figure 7 The recapitulated systemic extra-embryonic functions, that is, the coelomic-amniotic and the trophoblastic-yolk sac (or vitellum), are located into the space of Disse.** This fact results in the development of hepatic steatosis and steatohepatitis in prehepatic portal hypertensive rats. A: Amnion; ANS: Autonomic nervous system; BD: Bile duct; BI: Blood islands; CNS: Central nervous system; EM: Extraembryonic mesoderm; HA: Hepatic artery; HS: Steatotic liver; HSC: Hematological stem cell; HV: Hepatic vein; MSC: Mesenchymal stem cell; TPVL: Triple partial portal vein ligation; Y: Yolk sac.

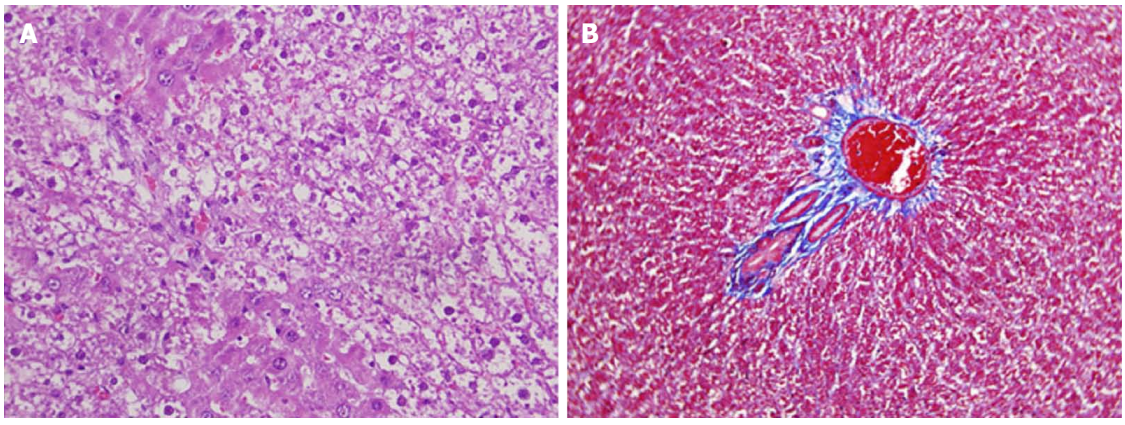


**Figure 8 Histological appearance of non-alcoholic steatohepatitis in portal hypertensive rats at three months of postoperative evolution.** It's shown the hepatocyte ballooning (A) with lobular inflammation and perisinuosidal fibrosis (B) (H and E stain, × 200).

tion, the coelomic-amniotic and trophoblastic-yolk sac functions are integrated into the interstitial space of Disse, thereby activating embryonic programs in the acinus. In this line, coelomic-amniotic functions would be embodied by the systemic neurogenic inflammatory phenotype, while the trophoblastic-yolk sac functions could be represented by the immune inflammatory phenotype[88] (Figure 6). Furthermore, the polarization of neurogenic and immune-related phenotypes in the interstitial space of Disse would condition the evolution of NAFLD, including a wound-healing response producing fibrosis in the rat[88,89] (Figure 4 and Table 1).

Essentially, the recapitulation of the extra-embryonic functions when focused on the space of Disse[88] would produce a gastrulation-like process which is a recapitulation of the intra-embryonic mesenchyme formation process[89]. Therefore, mesoderm-derived cells, particularly fibrocytes and hepatic stellate cells have shown a main role in liver restore (Figure 5) after TPVL. The involution or dedifferentiation of the liver in portal hypertensive rats could be exemplifying a prenatal specialization[88] which involves or angiogenic regenerative or fibrotic/scarring repairing processes[89].

The systemic complications of the prehepatic portal

hypertensive syndrome in rats could be connected to similar metabolic functions of the extra-embryonic coelomic-amniotic and trophoblastic-yolk sac axes[88]. For example, hydroelectrolytic decompensation and its effects, including hyperdynamic circulation, multiple neuroendocrine axis activation, edema due to sodium and water accumulation, increase interstitial hepato-intestinal lymph flow and stimulation of the sympathetic nervous system, might be associated to the upregulation of a systemic coelomic-amniotic axis after TPVL in the rat (Figure 7). In this line, an upregulation of a systemic trophoblastic-yolk sac or vitellogenic-like axis could be represented through the immune phenotype activation of NF-κB and inflammasome, acute phase response, splanchnic infiltration by mast cells, bacterial intestinal translocation which implies hepatic dyslipidaemia and the excessive splanchnic angiogenic response. The convergence of these systemic extraembryonic axes in the interstitial space of Disse could favor a gastrulation-like response in which NAFLD develops (Figure 7).

Recently, it has been established that human NAFLD is commonly associated with hypertension, type 2 diabetes, obesity, dyslipidemia, metabolic syndrome and cardiovascular abnormalities leading to death[90-92]. The array of alterations associated with the evolution of NAFLD make up a syndrome of great pathophysiological complexity. Studying this syndrome would help the experimental model of liver steatosis in the rat after TPVL. Also, the results obtained from studying multiple splanchnic and systemic alterations produced in the surgical experimental model of NAFLD suggest that the systemic inflammatory response could condition the evolution of hepatic steatosis. Thus, when the animals are isolated in individual cages after TPVL, accelerating the development of the liver histopathological changes typical of steatosis is possible (Figure 8). This early appearance of liver steatosis in TPVL-rats could be attributed to stress induced by isolation when rats are separated from other rats, and to their limited space for movement when kept in individual holders. If the stress related to isolation and movement limitation favors liver steatosis development, studying the influence of systemic neuroendocrine alterations associated with stress in NAFLD development in rats with TPVL would be an attractive goal for the future.

Finally, if our hypothesis presented in the current review of the NAFLD model secondary to TPVL in the rat represents the focus of two systemic functional axes that recapitulate extra-embryonic functions in the interstitial space of Disse, then modulating the evolution of experimental liver steatosis is possible by modifying the re-expression of these functions. For that reason, studying the mechanisms involved in embryonic development could provide key results for a better understanding of the NAFLD etiopathogeny.

## ACKNOWLEDGMENTS

The excellent secretarial support of Mrs. Maria-Elena Vicente is gratefully acknowledged. We would also like to thank Elizabeth Mascola for translating it into English and Javier de Jorge and Maria-José Valdemoro, Director and librarian, respectively of the Complutense University Medical School Library. This review was made, in part, thanks to FICYT FC-15-GRUPIN 14-088 and MINECO PSI2013-45924-P grants. Finally, Natalia Arias is now granted for Alfonso Martin Escudero Foundation.

## REFERENCES

1 **Brunt EM**. Pathology of nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 195-203 [PMID: 20195271 DOI: 10.1038/nrgastro.2010.21]
2 **Bhala N**, Angulo P, van der Poorten D, Lee E, Hui JM, Saracco G, Adams LA, Charatcharoenwitthaya P, Topping JH, Bugianesi E, Day CP, George J. The natural history of nonalcoholic fatty liver disease with advanced fibrosis or cirrhosis: an international collaborative study. *Hepatology* 2011; **54**: 1208-1216 [PMID: 21688282 DOI: 10.1002/hep.24491]
3 **Cohen JC**, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. *Science* 2011; **332**: 1519-1523 [PMID: 21700865 DOI: 10.1126/science.1204265]
4 **Townsend SA**, Newsome PN. Non-alcoholic fatty liver disease in 2016. *Br Med Bull* 2016; **119**: 143-156 [PMID: 27543499 DOI: 10.1093/bmb/ldw031]
5 **Henao-Mejia J**, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 2012; **482**: 179-185 [PMID: 22297845 DOI: 10.1038/nature10809]
6 **Takahashi Y**, Soejima Y, Fukusato T. Animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* 2012; **18**: 2300-2308 [PMID: 22654421 DOI: 10.3748/wjg.v18.i19.2300]
7 **Asaoka Y**, Terai S, Sakaida I, Nishina H. The expanding role of fish models in understanding non-alcoholic fatty liver disease. *Dis Model Mech* 2013; **6**: 905-914 [PMID: 23720231 DOI: 10.1242/dmm.011981]
8 **Alonso MJ**, Aller MA, Corcuera MT, Nava MP, Gómez F, Angulo A, Arias J. Progressive hepatocytic fatty infiltration in rats with prehepatic portal hypertension. *Hepatogastroenterology* 2005; **52**: 541-546 [PMID: 15816474]
9 **Prieto I**, Jiménez F, Aller MA, Nava MP, Vara E, Garcia C, Arias J. Tumor necrosis factor-alpha, interleukin-1beta and nitric oxide: induction of liver megamitochondria in prehepatic portal hypertensive rats. *World J Surg* 2005; **29**: 903-908 [PMID: 15951934 DOI: 10.1007/s00268-005-7757-5]
10 **Aller MA**, Vara E, García C, Nava MP, Angulo A, Sánchez-Patán F, Calderón A, Vergara P, Arias J. Hepatic lipid metabolism changes in short- and long-term prehepatic portal hypertensive rats. *World J Gastroenterol* 2006; **12**: 6828-6834 [PMID: 17106932]
11 **Izzet T**, Osman K, Ethem U, Nihat Y, Ramazan K, Mustafa D, Hafize U, Riza KA, Birsen A, Habibe G, Seval A, Gonul S. Oxidative stress in portal hypertension-induced rats with particular emphasis on nitric oxide and trace metals. *World J Gastroenterol* 2005; **11**: 3570-3573 [PMID: 15962377]
12 **Puoti C**, Bellis L. Steatosis and portal hypertension. *Eur Rev Med Pharmacol Sci* 2005; **9**: 285-290 [PMID: 16231591]
13 **Aller MA**, Mendez M, Nava MP, Lopez L, Curras D, De Paz A, Arias JL, Arias J. Microsurgery in Liver Research. Bentham e-Books, 2009: 117-136 [DOI: 10.2174/97816080506801090101]
14 **Ingber DE**. Cellular mechanotransduction: putting all the pieces together again. *FASEB J* 2006; **20**: 811-827 [PMID: 16675838 DOI: 10.1096/fj.05-5424rev]
15 **Wong VW**, Longaker MT, Gurtner GC. Soft tissue mechanotransduction in wound healing and fibrosis. *Semin Cell Dev*

*Biol* 2012; **23**: 981-986 [PMID: 23036529 DOI: 10.1016/j.semcdb.2012.09.010]
16 **Shrader CD**, Ressetar HG, Luo J, Cilento EV, Reilly FD. Acute stretch promotes endothelial cell proliferation in wounded healing mouse skin. *Arch Dermatol Res* 2008; **300**: 495-504 [PMID: 18330587 DOI: 10.1007/s00403-008-0836-3]
17 **Aller MA**, Arias JL, Nava MP, Arias J. Posttraumatic inflammation is a complex response based on the pathological expression of the nervous, immune, and endocrine functional systems. *Exp Biol Med* (Maywood) 2004; **229**: 170-181 [PMID: 14734796]
18 **Aller MA**, Arias JI, Prieto I, Gilsanz C, Arias A, Yang H, Arias J. Surgical inflammatory stress: the embryo takes hold of the reins again. *Theor Biol Med Model* 2013; **10**: 6 [PMID: 23374964 DOI: 10.1186/1742-4682-10-6]
19 **Aller MA**, Arias JL, Cruz A, Arias J. Inflammation: a way to understanding the evolution of portal hypertension. *Theor Biol Med Model* 2007; **4**: 44 [PMID: 17999758 DOI: 10.1186/1742-4682-4-44]
20 **García-Pagán JC**, Gracia-Sancho J, Bosch J. Functional aspects on the pathophysiology of portal hypertension in cirrhosis. *J Hepatol* 2012; **57**: 458-461 [PMID: 22504334 DOI: 10.1016/j.jhep.2012.03.007]
21 **Iwakiri Y**, Groszmann RJ. The hyperdynamic circulation of chronic liver diseases: from the patient to the molecule. *Hepatology* 2006; **43**: S121-S131 [PMID: 16447289 DOI: 10.1002/hep.20993]
22 **Bosch J**, Groszmann RJ, Shah VH. Evolution in the understanding of the pathophysiological basis of portal hypertension: How changes in paradigm are leading to successful new treatments. *J Hepatol* 2015; **62**: S121-S130 [PMID: 25920081 DOI: 10.1016/j.jhep.2015.01.003]
23 **Song D**, Liu H, Sharkey KA, Lee SS. Hyperdynamic circulation in portal-hypertensive rats is dependent on central c-fos gene expression. *Hepatology* 2002; **35**: 159-166 [PMID: 11786972 DOI: 10.1053/jhep.2002.30417]
24 **Liu H**, Schuelert N, McDougall JJ, Lee SS. Central neural activation of hyperdynamic circulation in portal hypertensive rats depends on vagal afferent nerves. *Gut* 2008; **57**: 966-973 [PMID: 18270244 DOI: 10.1136/gut.2007.135020]
25 **Frith J**, Newton JL. Autonomic dysfunction in chronic liver disease. *Hepat Med* 2011; **3**: 81-87 [PMID: 24367224 DOI: 10.2147/HMER.S16312]
26 **Arroyo V**, Moreau R, Jalan R, Ginès P. Acute-on-chronic liver failure: A new syndrome that will re-classify cirrhosis. *J Hepatol* 2015; **62**: S131-S143 [PMID: 25920082 DOI: 10.1016/j.jhep.2014.11.045]
27 **Chiu JJ**, Chien S. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. *Physiol Rev* 2011; **91**: 327-387 [PMID: 21248169 DOI: 10.1152/physrev.00047.2009]
28 **Schrier RW**, Arroyo V, Bernardi M, Epstein M, Henriksen JH, Rodés J. Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology* 1988; **8**: 1151-1157 [PMID: 2971015]
29 **Aller MA**, Arias N, Prieto I, Santamaria L, De Miguel MP, Arias JL, Arias J. Portal hypertension-related inflammatory phenotypes: From a vitelline and amniotic point of view. *Adv Biosci Biotechnol* 2012; **3**: 881-899 [DOI: 10.4236/abb.2012.37110]
30 **Yamasaki M**, Ikeda K, Nakatani K, Yamamoto T, Kawai Y, Hirohashi K, Kinoshita H, Kaneda K. Phenotypical and morphological alterations to rat sinusoidal endothelial cells in arterialized livers after portal branch ligation. *Arch Histol Cytol* 1999; **62**: 401-411 [PMID: 10678569]
31 **Lautt WW**. Mechanism and role of intrinsic regulation of hepatic arterial blood flow: hepatic arterial buffer response. *Am J Physiol* 1985; **249**: G549-G556 [PMID: 3904482]
32 **SCHAFFNER F**, POPER H. Capillarization of hepatic sinusoids in man. *Gastroenterology* 1963; **44**: 239-242 [PMID: 13976646]
33 **Xie G**, Wang X, Wang L, Wang L, Atkinson RD, Kanel GC, Gaarde WA, Deleve LD. Role of differentiation of liver sinusoidal endothelial cells in progression and regression of hepatic fibrosis in rats. *Gastroenterology* 2012; **142**: 918-927.e6 [PMID: 22178212 DOI: 10.1053/j.gastro.2011.12.017]
34 **Guidotti LG**, Inverso D, Sironi L, Di Lucia P, Fioravanti J, Ganzer L, Fiocchi A, Vacca M, Aiolfi R, Sammicheli S, Mainetti M, Cataudella T, Raimondi A, Gonzalez-Aseguinolaza G, Protzer U, Ruggeri ZM, Chisari FV, Isogawa M, Sitia G, Iannacone M. Immunosurveillance of the liver by intravascular effector CD8(+) T cells. *Cell* 2015; **161**: 486-500 [PMID: 25892224 DOI: 10.1016/j.cell.2015.03.005]
35 **Ohtani O**, Ohtani Y. Lymph circulation in the liver. *Anat Rec (Hoboken)* 2008; **291**: 643-652 [PMID: 18484610 DOI: 10.1002/ar.20681]
36 **Sawitza I**, Kordes C, Reister S, Häussinger D. The niche of stellate cells within rat liver. *Hepatology* 2009; **50**: 1617-1624 [PMID: 19725107 DOI: 10.1002/hep.23184]
37 **Kordes C**, Häussinger D. Hepatic stem cell niches. *J Clin Invest* 2013; **123**: 1874-1880 [PMID: 23635785 DOI: 10.1172/JCI66027]
38 **Lugrin J**, Rosenblatt-Velin N, Parapanov R, Liaudet L. The role of oxidative stress during inflammatory processes. *Biol Chem* 2014; **395**: 203-230 [PMID: 24127541 DOI: 10.1515/hsz-2013-0241]
39 **Awad EM**, Khan SY, Sokolikova B, Brunner PM, Olcaydu D, Wojta J, Breuss JM, Uhrin P. Cold induces reactive oxygen species production and activation of the NF-kappa B response in endothelial cells and inflammation in vivo. *J Thromb Haemost* 2013; **11**: 1716-1726 [PMID: 23865569 DOI: 10.1111/jth.12357]
40 **Kleniewska P**, Piechota-Polanczyk A, Michalski L, Michalska M, Balcerczak E, Zebrowska M, Goraca A. Influence of block of NF-kappa B signaling pathway on oxidative stress in the liver homogenates. *Oxid Med Cell Longev* 2013; **2013**: 308358 [PMID: 23577221 DOI: 10.1155/2013/308358]
41 **Gabay C**, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999; **340**: 448-454 [PMID: 9971870 DOI: 10.1056/NEJM199902113400607]
42 **Cray C**, Zaias J, Altman NH. Acute phase response in animals: a review. *Comp Med* 2009; **59**: 517-526 [PMID: 20034426]
43 **Aller MA**, Arias JL, Arias J. The mast cell integrates the splanchnic and systemic inflammatory response in portal hypertension. *J Transl Med* 2007; **5**: 44 [PMID: 17892556 DOI: 10.1186/1479-5876-5-44]
44 **Diez-Arias JA**, Aller MA, Palma MD, Arias JL, Muñiz E, Sánchez M, Arias J. Increased duodenal mucosa infiltration by mast cells in rats with portal hypertension. *Dig Surg* 2001; **18**: 34-40 [PMID: 11244257]
45 **Prieto I**, Aller MA, Santamaría L, Nava MP, Madero R, Pérez-Robledo JP, Arias J. Prehepatic portal hypertension produces increased mast cell density in the small bowel and in mesenteric lymph nodes in the rat. *J Gastroenterol Hepatol* 2005; **20**: 1025-1031 [PMID: 15955210 DOI: 10.1111/j.1440-1746.2005.03831.x]
46 **Moquillaza LM**, Aller MA, Nava MP, Santamaría L, Vergara P, Arias J. Partial hepatectomy, partial portal vein stenosis and mesenteric lymphadenectomy increase splanchnic mast cell infiltration in the rat. *Acta Histochem* 2010; **112**: 372-382 [PMID: 19446312 DOI: 10.1016/j.acthis.2009.03.002]
47 **Merino J**, Aller MA, Rubio S, Arias N, Nava MP, Loscertales M, Arias J, Arias JL. Gut-brain chemokine changes in portal hypertensive rats. *Dig Dis Sci* 2011; **56**: 2309-2317 [PMID: 21347560 DOI: 10.1007/s10620-011-1625-y]
48 **Aller MA**, Heras N, Blanco-Rivero J, Arias JI, Lahera V, Balfagón G, Arias J. Portal hypertensive cardiovascular pathology: the rescue of ancestral survival mechanisms? *Clin Res Hepatol Gastroenterol* 2012; **36**: 35-46 [PMID: 22264837 DOI: 10.1016/j.clinre.2011.07.017]
49 **de Las Heras N**, Aller MA, Martín-Fernández B, Miana M, Ballesteros S, Regadera J, Cachofeiro V, Arias J, Lahera V. A wound-like inflammatory aortic response in chronic portal hypertensive rats. *Mol Immunol* 2012; **51**: 177-187 [PMID: 22463791 DOI: 10.1016/j.molimm.2012.03.016]
50 **De las Heras N**, Aller MA, Arias J, Lahera V. The risk association between experimental portal hypertension and an aortic atherosclerosis-like disease. *Hepatology* 2013; **57**: 421-422 [PMID: 22730048 DOI: 10.1002/hep.25917]
51 **Sánchez-Patán F**, Anchuelo R, Aller MA, Vara E, García C, Nava MP, Arias J. Chronic prehepatic portal hypertension in the rat: is it a type of metabolic inflammatory syndrome? *Lipids Health Dis* 2008;

**7**: 4 [PMID: 18271959 DOI: 10.1186/1476-511X-7-4]
52 **Kim LJ**, Nalls MA, Eiriksdottir G, Sigurdsson S, Launer LJ, Koster A, Chaves PH, Jonsdottir B, Garcia M, Gudnason V, Harris TB. Associations of visceral and liver fat with the metabolic syndrome across the spectrum of obesity: the AGES-Reykjavik study. *Obesity (Silver Spring)* 2011; **19**: 1265-1271 [PMID: 21183935 DOI: 10.1038/oby.2010.291]
53 **Tufail M**, Takeda M. Molecular characteristics of insect vitellogenins. *J Insect Physiol* 2008; **54**: 1447-1458 [PMID: 18789336 DOI: 10.1016/j.jinsphys.2008.08.007]
54 **Arukwe A**, Goksøyr A. Eggshell and egg yolk proteins in fish: hepatic proteins for the next generation: oogenetic, population, and evolutionary implications of endocrine disruption. *Comp Hepatol* 2003; **2**: 4 [PMID: 12685931]
55 **Targher G**, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *N Engl J Med* 2010; **363**: 1341-1350 [PMID: 20879883 DOI: 10.1056/NEJMra0912063]
56 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102]
57 **García-Ruiz C**, Baulies A, Mari M, García-Rovés PM, Fernandez-Checa JC. Mitochondrial dysfunction in non-alcoholic fatty liver disease and insulin resistance: cause or consequence? *Free Radic Res* 2013; **47**: 854-868 [PMID: 23915028 DOI: 10.3109/10715762.2013.830717]
58 **Oh MK**, Winn J, Poordad F. Review article: diagnosis and treatment of non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2008; **28**: 503-522 [PMID: 18532991 DOI: 10.1111/j.1365-2036.2008.03752.x]
59 **Hollenberg SM**, Waldman B. The Circulatory System in Liver Disease. *Crit Care Clin* 2016; **32**: 331-342 [PMID: 27339674 DOI: 10.1016/j.ccc.2016.02.004]
60 **Wiernsperger N**. Hepatic function and the cardiometabolic syndrome. *Diabetes Metab Syndr Obes* 2013; **6**: 379-388 [PMID: 24143116 DOI: 10.2147/DMSO.S51145]
61 **Coulon S**, Heindryckx F, Geerts A, Van Steenkiste C, Colle I, Van Vlierberghe H. Angiogenesis in chronic liver disease and its complications. *Liver Int* 2011; **31**: 146-162 [PMID: 21073649 DOI: 10.1111/j.1478-3231.2010.02369.x]
62 **Thabut D**, Shah V. Intrahepatic angiogenesis and sinusoidal remodeling in chronic liver disease: new targets for the treatment of portal hypertension? *J Hepatol* 2010; **53**: 976-980 [PMID: 20800926 DOI: 10.1016/j.jhep.2010.07.004]
63 **Viggiano TR**, Gostout CJ. Portal hypertensive intestinal vasculopathy: a review of the clinical, endoscopic, and histopathologic features. *Am J Gastroenterol* 1992; **87**: 944-954 [PMID: 1642217]
64 **Mekaroonkamol P**, Cohen R, Chawla S. Portal hypertensive enteropathy. *World J Hepatol* 2015; **7**: 127-138 [PMID: 25729469 DOI: 10.4254/wjh.v7.i2.127]
65 **Fernandez M**, Mejias M, Angermayr B, Garcia-Pagan JC, Rodés J, Bosch J. Inhibition of VEGF receptor-2 decreases the development of hyperdynamic splanchnic circulation and portal-systemic collateral vessels in portal hypertensive rats. *J Hepatol* 2005; **43**: 98-103 [PMID: 15893841 DOI: 10.1016/j.jhep.2005.02.022]
66 **Angermayr B**, Mejias M, Gracia-Sancho J, Garcia-Pagan JC, Bosch J, Fernandez M. Heme oxygenase attenuates oxidative stress and inflammation, and increases VEGF expression in portal hypertensive rats. *J Hepatol* 2006; **44**: 1033-1039 [PMID: 16458992 DOI: 10.1016/j.jhep.2005.09.021]
67 **Shaik-Dasthagirisaheb YB**, Varvara G, Murmura G, Saggini A, Potalivo G, Caraffa A, Antinolfi P, Tete' S, Tripodi D, Conti F, Cianchetti E, Toniato E, Rosati M, Conti P, Speranza L, Pantalone A, Saggini R, Theoharides TC, Pandolfi F. Vascular endothelial growth factor (VEGF), mast cells and inflammation. *Int J Immunopathol Pharmacol* 2013; **26**: 327-335 [PMID: 23755748]
68 **Friedman SL**. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev* 2008; **88**: 125-172 [PMID: 18195085 DOI: 10.1152/physrev.00013.2007]
69 **Parola M**, Pinzani M. Hepatic wound repair. *Fibrogenesis Tissue Repair* 2009; **2**: 4 [PMID: 19781064 DOI: 10.1186/1755-1536-2-4]
70 **Parker GA**, Picut CA. Immune functioning in non lymphoid organs: the liver. *Toxicol Pathol* 2012; **40**: 237-247 [PMID: 22089842 DOI: 10.1177/0192623311428475]
71 **Hellerbrand C**. Hepatic stellate cells--the pericytes in the liver. *Pflugers Arch* 2013; **465**: 775-778 [PMID: 23292551 DOI: 10.1007/s00424-012-1209-5]
72 **Reynaert H**, Urbain D, Geerts A. Regulation of sinusoidal perfusion in portal hypertension. *Anat Rec* (Hoboken) 2008; **291**: 693-698 [PMID: 18484616 DOI: 10.1002/ar.20669]
73 **Kasuya J**, Sudo R, Mitaka T, Ikeda M, Tanishita K. Spatio-temporal control of hepatic stellate cell-endothelial cell interactions for reconstruction of liver sinusoids in vitro. *Tissue Eng Part A* 2012; **18**: 1045-1056 [PMID: 22220631 DOI: 10.1089/ten.TEA.2011.0351]
74 **Jauniaux E**, Gulbis B. Fluid compartments of the embryonic environment. *Hum Reprod Update* 2000; **6**: 268-278 [PMID: 10874572]
75 **Burton GJ**, Hempstock J, Jauniaux E. Nutrition of the human fetus during the first trimester--a review. *Placenta* 2001; **22** Suppl A: S70-S77 [PMID: 11312634 DOI: 10.1053/plac.2001.0639]
76 **Jauniaux E**, Gulbis B, Jurkovic D, Campbell S, Collins WP, Ooms HA. Relationship between protein concentrations in embryological fluids and maternal serum and yolk sac size during human early pregnancy. *Hum Reprod* 1994; **9**: 161-166 [PMID: 8195341]
77 **Chang YJ**, Hwang SM, Tseng CP, Cheng FC, Huang SH, Hsu LF, Hsu LW, Tsai MS. Isolation of mesenchymal stem cells with neurogenic potential from the mesoderm of the amniotic membrane. *Cells Tissues Organs* 2010; **192**: 93-105 [PMID: 20215735 DOI: 10.1159/000295774]
78 **Uberti MG**, Pierpont YN, Ko F, Wright TE, Smith CA, Cruse CW, Robson MC, Payne WG. Amnion-derived cellular cytokine solution (ACCS) promotes migration of keratinocytes and fibroblasts. *Ann Plast Surg* 2010; **64**: 632-635 [PMID: 20395817 DOI: 10.1097/SAP.0b013e3181c39351]
79 **Bellini C**, Boccardo F, Bonioli E, Campisi C. Lymphodynamics in the fetus and newborn. *Lymphology* 2006; **39**: 110-117 [PMID: 17036631]
80 **Shaw SW**, David AL, De Coppi P. Clinical applications of prenatal and postnatal therapy using stem cells retrieved from amniotic fluid. *Curr Opin Obstet Gynecol* 2011; **23**: 109-116 [PMID: 21386681 DOI: 10.1097/GCO.0b013e32834457b1]
81 **Fraser ST**, Baron MH. Embryonic fates for extraembryonic lineages: New perspectives. *J Cell Biochem* 2009; **107**: 586-591 [PMID: 19415688 DOI: 10.1002/jcb.22165]
82 **Gulbis B**, Jauniaux E, Cotton F, Stordeur P. Protein and enzyme patterns in the fluid cavities of the first trimester gestational sac: relevance to the absorptive role of secondary yolk sac. *Mol Hum Reprod* 1998; **4**: 857-862 [PMID: 9783845]
83 **Siegel N**, Rosner M, Hanneder M, Freilinger A, Hengstschläger M. Human amniotic fluid stem cells: a new perspective. *Amino Acids* 2008; **35**: 291-293 [PMID: 17710362 DOI: 10.1007/s00726-007-0593-1]
84 **Ueno H**, Weissman IL. The origin and fate of yolk sac hematopoiesis: application of chimera analyses to developmental studies. *Int J Dev Biol* 2010; **54**: 1019-1031 [PMID: 20711980 DOI: 10.1387/ijdb.093039hu]
85 **Yoshida S**, Wada Y. Transfer of maternal cholesterol to embryo and fetus in pregnant mice. *J Lipid Res* 2005; **46**: 2168-2174 [PMID: 16061954 DOI: 10.1194/jlr.M500096-JLR200]
86 **Nakazawa F**, Alev C, Jakt LM, Sheng G. Yolk sac endoderm is the major source of serum proteins and lipids and is involved in the regulation of vascular integrity in early chick development. *Dev Dyn* 2011; **240**: 2002-2010 [PMID: 21761483 DOI: 10.1002/dvdy.22690]
87 **Bevilacqua E**, Hoshida MS, Amarante-Paffaro A, Albieri-Borges A, Zago Gomes S. Trophoblast phagocytic program: roles in different placental systems. *Int J Dev Biol* 2010; **54**: 495-505 [PMID: 19757392 DOI: 10.1387/ijdb.082761eb]
88 **Aller MA**, Blanco-Rivero J, Arias JI, Balfagon G, Arias J. The wound-healing response and upregulated embryonic mechanisms: brothers-in-arms forever. *Exp Dermatol* 2012; **21**: 497-503 [PMID: 22716244 DOI: 10.1111/j.1600-0625.2012.01525.x]

89 **Aller MA**, Arias JI, Arias J. Pathological axes of wound repair: gastrulation revisited. *Theor Biol Med Model* 2010; **7**: 37 [PMID: 20840764 DOI: 10.1186/1742-4682-7-37]
90 **Hyogo H**, Chayama K, Yamagishi S. Nonalcoholic fatty liver disease and cardiovascular disease. *Curr Pharm Des* 2014; **20**: 2403-2411 [PMID: 23844815]
91 **Tarantino G**, Finelli C. What about non-alcoholic fatty liver disease as a new criterion to define metabolic syndrome? *World J Gastroenterol* 2013; **19**: 3375-3384 [PMID: 23801829 DOI: 10.3748/wjg.v19.i22.3375]
92 **Wang Y**, Li YY, Nie YQ, Zhou YJ, Cao CY, Xu L. Association between metabolic syndrome and the development of non-alcoholic fatty liver disease. *Exp Ther Med* 2013; **6**: 77-84 [PMID: 23935723 DOI: 10.3892/etm.2013.1090]

**P- Reviewer**: Dourakis SP, Manenti A, Popovic DD, Zapater P
**S- Editor**: Ji FF **L- Editor**: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pathophysiology

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

World J Gastrointest Pathophysiol 2017 May 15; 8(2): 51-58

DOI: 10.4291/wjgp.v8.i2.51

ISSN 2150-5330 (online)

MINIREVIEWS

# Non-alcoholic fatty liver disease and cardiovascular risk

Rashmee Patil, Gagan K Sood

Rashmee Patil, Gagan K Sood, Division of Gastroenterology and Hepatology, Baylor College of Medicine, St. Luke's Liver Center, Baylor Clinic, Houston, TX 77030, United States

Author contributions: Patil R and Sood GK were involved in designing and writing the manuscript; Patil R was responsible for a thorough literature search; Sood GK was involved in editing the manuscript.

Conflict-of-interest statement: Patil R and Sood GK have no conflicts of interest to report.



Manuscript source: Invited manuscript

Correspondence to: Gagan K Sood, MD, Division of Gastroenterology and Hepatology, Baylor College of Medicine, St. Luke's Liver Center, 6620 Main Street, Suite 1425, Baylor Clinic, Houston, TX 77030, United States. gksood@bcm.edu
Telephone: +1-832-3551400

Received: August 27, 2016
Peer-review started: August 31, 2016
First decision: December 13, 2016
Revised: December 30, 2016
Accepted: March 12, 2017
Article in press: March 13, 2017
Published online: May 15, 2017

## Abstract

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease associated with insulin resistance and metabolic syndrome. The spectrum of disease ranges from simple steatosis to steatohepatitis and progression to cirrhosis. Compelling evidence over the past several years has substantiated a significant link between NAFLD and cardiovascular disease ranging from coronary artery disease to subclinical carotid atherosclerosis. Close follow up, treatment of risk factors for NAFLD, and cardiovascular risk stratification are necessary to predict morbidity and mortality in this subset of patients.

**Key words:** Non-alcoholic fatty liver disease; Cardiovascular risk; Outcomes; Coronary artery disease; Steatosis



**Core tip:** Non-alcoholic fatty liver disease (NAFLD) is often associated with insulin resistance and is strongly associated with type 2 diabetes mellitus and obesity. In addition to being at risk for nonalcoholic steatohepatitis, cirrhosis and its complications, NAFLD patients are also at higher risk of cardiovascular diseases (CVD), including coronary heart disease and stroke. NAFLD confers increased cardiovascular disease risk independent of traditional cardiovascular risk factors and metabolic syndrome. Close followup of patients with NAFLD may be indicated to prevent major vascular events. Risk stratification scores are needed that address both the risk for advanced liver disease and CVD.

Patil R, Sood GK. Non-alcoholic fatty liver disease and cardiovascular risk. *World J Gastrointest Pathophysiol* 2017; 8(2): 51-58 Available from: URL: http://www.wjgnet.com/2150-5330/full/v8/i2/51.htm DOI: http://dx.doi.org/10.4291/wjgp.v8.i2.51

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the upcoming leading cause of chronic liver disease in the United States and its prevalence is increasing world-

wide. It is a spectrum of liver diseases that ranges from simple steatosis to a progressive form of liver disease called nonalcoholic steatohepatitis (NASH)[1]. It may progress to advanced fibrosis, cirrhosis, and hepatocellular carcinoma in some individuals. NAFLD is often associated with insulin resistance and is strongly associated with type 2 diabetes mellitus and obesity. NAFLD patients are at risk of progressing to NASH and ultimately cirrhosis; they are also at higher risk of cardiovascular diseases (CVD), including coronary heart disease and stroke[2]. NAFLD confers increased cardiovascular disease risk independent of traditional cardiovascular risk factors and metabolic syndrome (MetS). In this review, we have discussed the association of NAFLD with cardiovascular disease, the likely mechanisms underlying this association, proper risk assessment of patients with NAFLD for cardiovascular diseases, and treatment options for modification of CVD morbidity and mortality in patients with NAFLD.

## EPIDEMIOLOGY OF CVD IN NAFLD

Adverse CVD events in NAFLD subjects compared with the general population are described through a description of several recent epidemiological studies (Table 1). The diagnosis of NAFLD can be based either on histology or imaging studies. Abnormal liver enzymes are used as biochemical surrogates of NAFLD in several recent studies. The prospective cohort study by Dunn *et al*[3], using data from the Third National Health and Nutrition Examination Survey (NHANES Ⅲ), showed that subjects with NAFLD identified based on elevated alanine aminotransferase (ALT) had a higher mortality from CVD. After adjusting for cardiovascular risk factors, several large population studies have shown an association between an elevated ALT and increased cardiovascular mortality[4,5]. Elevated GGT has also been reported as a marker of NAFLD in described prospective studies. A meta-analysis of ten pooled studies confirmed the independent association between elevated GGT and adverse CV events[6].

Liver imaging may be a more reliable method for diagnosing NAFLD. In three large population studies, ultrasound imaging suggestive of NAFLD was independently associated with cardiovascular events[7-9]. Although these studies did show that NAFLD may be a predictor of CVD, ultrasound may not be as sensitive for a diagnosis of NAFLD and therefore this was a major limitation for these studies. Hepatic fat concentration as measured by MRI has been used for diagnosis of NAFLD. Quantity of liver fat has been reported to be predictive of metabolic syndrome and CVD risk. In a recent study Loomba *et al*[10] quantified liver fat in patients with NAFLD and controls using a magnetic resonance imaging and measuring proton-density-fat-fraction (MRI-PDFF). In patients with NAFLD high proton-density-fat-fraction on MRI was a predictor of metabolic syndrome and increased cardiovascular risk[10].

Liver biopsy is considered to be the gold standard for diagnosis of NAFLD and some studies have shown that patients with biopsy-proven NAFLD have higher total mortality rates compared to the general population. Söderberg *et al*[11] reported increased mortality from cardiovascular disease in patients with biopsy proven NASH. Forty-three percent of patients had NASH at initial biopsy. The median follow up in their study was 28 years. Overall survival was reduced in subjects with NASH compared to the general population due to increased mortality by cardiovascular disease. Importantly in this study, only subjects with NASH had significantly reduced survival[9-11].

## MECHANISM (PATHOGENESIS)

Several mechanisms have been postulated for development of accelerated atherosclerosis in patients with NAFLD, including genetic predisposition, insulin resistance and atherogenic dyslipidemia, oxidative stress, chronic inflammation, reduced levels of the adiponectin and altered production of pro and anticoagulant factors[12]. All these mechanisms are present at the same time. NAFLD, regardless of its stage, is strongly associated with hepatic and adipose tissue insulin resistance (IR). In fact, liver fat content can be used as an independent predictor of insulin resistance. These mechanisms work synergistically[13].

NAFLD, especially in its necroinflammatory form (NASH), may cause atherogenic dyslipidemia[14]. In addition there is an increase of pro-coagulant factors like fibrinogen, plasminogen activator inhibitor-1 and tumor growth factor, which all increase the risk of atherosclerosis[15]. NAFLD is considered to have chronic sub-clinical inflammation and associated with many inflammatory markers. Increased vascular risk has been linked to increased levels of inflammatory cytokines and markers such as IL-6, TNF, CRP, and fibrinogen. Oxidative stress may also play a role. This stress is thought to trigger changes in endothelial function leading to formation and deposition of oxidized LDL in the sub-intimal space[16].

Visceral adipose tissue is also thought to play a role in NAFLD. Visceral fat is metabolically active and secretes several hormones that help regulate inflammation; tissue distribution is affected by an alteration in cellular free fatty acid transport. These alterations are possibly caused by hyperinsulinemia and ultimately divert accumulated triglycerides away from adipose tissue and towards other metabolic organs such as the liver[17,18].

Nonalcoholic fatty liver disease, abdominal obesity, and insulin resistance all play a role in increased cardiovascular risk, though the exact causal relationship is still unclear. Hepatic necroinflammation, as seen in NASH, is an atherogenic mechanism that may explain why patients with NASH have greater CV risk than patients with simple steatosis. In the liver, a signal of hepatic necroinflammation is elevated liver enzymes which may

**Table 1 Recent epidemiological studies evaluating cardiovascular risk in non-alcoholic fatty liver disease**

| Ref. | Study characteristics | Years of follow-up | Diagnosis of NAFLD | Study outcomes | Main findings |
|---|---|---|---|---|---|
| Ekstedt *et al*[30] (2015) | Retrospective cohort study *n* = 229 Swedish patients with NAFLD and elevated liver enzymes (49% NASH); mean age 49 yr, 66% men | 26.4 (mean) | Histology | *n* = 96 total deaths, 41 CVD related deaths | Increased rates of all-cause, liver-related and CVD mortality with NAFLD compared with general control population. Fibrosis stage on histology significantly predicted the risk of all-cause, liver-related and CVD mortality |
| Ekstedt *et al*[31] (2006) | Cohort study 129 consecutively enrolled patients diagnosed with biopsy-proven NAFLD were reevaluated. Survival and causes of death were compared with a matched reference population. Living NAFLD patients were offered repeat liver biopsy and clinical and biochemical investigation | 13.7 (mean) | Histology | Mortality was not increased in patients with steatosis. Survival of patients with nonalcoholic steatohepatitis (NASH) was reduced. These subjects more often died from cardiovascular and liver-related causes. At follow-up, 69 of 88 patients had diabetes or impaired glucose tolerance. Progression of liver fibrosis occurred in 41%. These subjects more often had a weight gain exceeding 5 kg, they were more insulin resistant, and they exhibited more pronounced hepatic fatty infiltration at follow-up | Increased total mortality which was primarily CV related (only in NASH patients but not in simple steatosis) compared with matched reference population |
| Soderberg *et al*[11] (2010) | Retrospective cohort study 256 subjects (61% men, mean age of 45 ± 12 yr) This study was undertaken to determine the frequency of NAFLD in a cohort of subjects who underwent liver biopsy from 1980 to 1984 because of elevated liver enzymes, and to assess mortality among subjects with NAFLD in comparison with the general Swedish population. Liver biopsies were blindly scored for NAFLD and NASH | 24 yr (mean) | Histology | During the follow-up period, 113 (44%) of the total population and 47 (40%) of the 118 subjects diagnosed with NAFLD died. Of the 113 deaths, 37 were of cardiovascular disease and 16 of liver diseases. NAFLD exhibited a 69% increased mortality, subjects with bland steatosis, a 55% increase, and subjects with NASH, 86% | Increased total mortality in NAFLD was predominantly CV related, compared with matched reference population |
| Pickhardt *et al*[32] (2014) | Retrospective cohort study United States adults undergoing abdominal CT selected among 4412 consecutive adults scanned with CT for clinical reasons over a 12-mo period: 282 NAFLD patients and 786 non-steatotic controls after exclusion of those with known liver diseases or < 1 yr of follow-up; mean 51 yr, 46% men | 7.5 (mean) | Unenhanced CT | Non-fatal CVD events (myocardial infarction, stroke, TIA or coronary bypass or stent); *n* = 73 CVD events | NAFLD was not independently associated with non-fatal CVD events |
| Zeb *et al*[12] (2016) | Prospective cohort study *n* = 4119 United States participants aged 45-84 yr (mean 62 yr, 45% men) who were free of CVD and known liver diseases at baseline | 7.6 (mean) | Unenhanced CT | All-cause mortality and no-fatal CVD events (myocardial infarction, resuscitated cardiac arrest, angina, or coronary revascularization procedures), *n* = 253 deaths and 209 non-fatal CVD events | NAFLD was independently associated with a composite endpoint inclusive of all-cause death and non-fatal CVD events |

| | | | | | |
|---|---|---|---|---|---|
| Kim *et al*[33] (2013) | Population-based cohort *n* = 11154 Unites States adults; mean age 43 yr, 48% men | 14.5 (median) | Ultrasound | All-cause and CVD mortality *n* = 1795 total deaths (673 CVD deaths) | NAFLD was not associated with increased all-cause and CVD mortality in the whole cohort however NAFLD with advanced fibrosis (defined by the NAFLD fibrosis score) was independently associated with increased all-cause and CVD mortality |
| Emre *et al*[34] (2015) | Retrospective cohort study *n* = 186 Turkish, non-diabetic patients undergoing PCI for ST-elevation MI; patients with known liver disease were excluded; mean age 58 yr, 78% men | In-hospital cardiac events | Ultrasound | In-hospital CVD events (MI, acute heart failure, cardiac arrest), *n* = 32 CVD events and *n* = 8 CVD deaths | Moderate-severe NAFLD was independently associated with increased in-hospital CVD events but not with increased CVD death |

NAFLD: Non-alcoholic fatty liver disease; CT: Computed tomography; CAC: Coronary artery calcification; CIMT: Carotid intima-media thickness; CP: Carotid plaque; DM: Diabetes mellitus; NASH: Nonalcoholic steatohepatitis.

serve as a marker for those at increased risk of CVD[19]. Patients with NASH are also noted in several studies to have a greatly increased carotid-artery intimal medial thickness which further supports the necroinflammation hypothesis.

Further research is required to uncover other specific mechanisms by which nonalcoholic fatty liver disease and nonalcoholic steatohepatitis may contribute to the development and progression of cardiovascular disease[20].

## EVIDENCE FOR CORONARY ARTERY DISEASE IN NAFLD

Atherosclerosis is the main trigger of overall vascular disease and different methods are used to detect it in its subclinical stage. Endothelial dysfunction is the first stage of subclinical atherosclerosis. Carotid intima-media thickness (CIMT) and the presence of carotid plaques are important markers of vascular disease. Other markers of atherosclerosis are coronary artery calcification (CAC), as determined by multi-slice CT scan. CAC represents the atherosclerotic burden in arterial beds and is known to correlate strongly with the presence of coronary artery disease (CAD) and increased risk of poor cardiovascular outcomes.

Several studies have demonstrated the association of coronary artery calcium score (CACS) with NAFLD (Table 2). A recent, large, population-based study reported a strong relationship between NAFLD and CAC. Importantly, this association was independent of the traditional risk factors for coronary artery disease[21]. Assy *et al*[21] described that the presence of NAFLD was associated with increased prevalence of non-calcified coronary plaques, independent of metabolic syndrome, in a case-controlled study. Another study showed a significant association in NAFLD patients and the appearance of vulnernable plaques on coronary artery imaging[22,23].

Though definitions of "significant" coronary artery disease may vary from study to study, a strong correlation exists between NAFLD and the prevalence of CAD as determined by coronary angiography.

## STUDIES EVALUATING SUBCLINICAL ATHEROSCLEROSIS IN NAFLD

Measuring carotid intima-media thickness (CIMT) by ultrasound is a widely accepted screening tool for the prediction of cardiovascular disease in patients with NAFLD who may be asymptomatic. Several studies show an association between NAFLD and carotid disease, some independently and other weakly after adjusting for metabolic syndrome. There seems to be a correlation histologically in severity of NAFLD when compared to increasing CIMT.

There is also evidence to support the association of NAFLD with subclinical atherosclerosis independent of traditional risk factors and metabolic syndrome. In a recent comprehensive systematic review there was strong evidence that NAFLD is associated with subclinical atherosclerosis[24]. The presence of NAFLD was associated with the increased severity of CIMT, coronary calcification, endothelial dysfunction and arterial stiffness. These were independent of traditional risk factors and metabolic syndrome.

## CARDIOVASCULAR RISK ASSESSMENT IN PATIENTS WITH NAFLD

All patients with NAFLD should be evaluated for CVD disease risk; this assessment can be repeated every 1-2 years. Patients should be evaluated for traditional CVD risk factors including obesity, diabetes, dyslipidemia and hypertension. Fasting glucose or glycosylated hemoglobin level should be done on the initial visit to diagnose DM. MetS is frequent in individuals with NAFLD and is associated with increased CVD and all-cause mortality. Therefore, assessment for the MetS is an important component of CV risk stratification. The MetS as defined by the National Cholesterol Education

**Table 2 Studies evaluating coronary artery disease and carotid disease in non-alcoholic fatty liver disease**

| Ref. | Study characteristics | Modality to assess CV risk | Diagnosis of NAFLD Ultrasound | Main findings |
|---|---|---|---|---|
| Sinn *et al*[16] (2016) | Retrospective cohort study - 8020 men (average age, 49.2 yr) without carotid atherosclerosis at baseline and with proven NAFLD | CIMT on carotid ultrasound | | NAFLD was associated with an increased risk of subclinical carotid atherosclerosis development. This association was explained by metabolic factors that could be potential mediators of the effect of NAFLD. Markers of liver fibrosis also were associated with subclinical carotid atherosclerosis development |
| Pais *et al*[35] (2016) | Longitudinal cohort study - 1871 subjects (mean age 53 yr; 65% males). Half of cohort had steatosis while half did not | CIMT on carotid ultrasound | Fatty Liver Index | Steatosis occurred in 12% and CP in 23% of patients. C-IMT increased in patients with steatosis occurrence whereas it did not change in those that stayed free of steatosis. Steatosis at baseline predicted CP occurrence independent of age, sex, type-2 diabetes, tobacco use, hsCRP, hypertension and C-IMT |
| Park *et al*[36] (2016) | Longitudinal cohort study - 1732 subjects underwent serial CAC evaluation. Half the cohort had NAFLD and half did not | Calcium scoring CT to assess CAC | Ultrasound | More subjects with NAFLD than without showed CAC development or progression. In subjects without calcification at baseline, NAFLD significantly affected the development of calcification after adjusting for traditional metabolic risk factors. The severity of NAFLD was dose-dependently associated with the development of CAC |
| Kim *et al*[15] (2012) | Retrospective chart review- 4 023 subjects (mean age, 56.9 ± 9.4 yr; 60.7% males) without known liver disease or a history of ischemic heart disease | Calcium scoring CT to assess CAC | Ultrasound | Patients with NAFLD are at increased risk for coronary atherosclerosis independent of classical coronary risk factors, including visceral adiposity. These data suggest that NAFLD might be an independent risk factor for coronary artery disease |
| Fracanzani *et al*[20] (2016) | Longitudinal cohort study - 125 NAFLD patients and 250 age and gender matched Controls at baseline and 10 yr later were followed. Incidence of cardiovascular and cerebral events was recorded | CIMT on carotid ultrasound | Ultrasound | Major cardiovascular events were observed in 19% of NAFLD patients, with an estimated cumulative risk significantly higher in NAFLD than in Controls. Presence of plaques and of steatosis were the strongest predictors for cardiovascular events. Grade of steatosis, ALT and GGT levels were higher in NAFLD patients who developed cardiovascular events. CIMT value after 10 years was significantly higher in NAFLD than in Controls. NAFLD should be included among risk factors for cardiovascular damage and underline the utility to evaluate, once it is diagnosed, the presence of atherosclerotic lesions |
| Nahandi *et al*[17] (2014) | Case control study - 151 patients in three groups: group I including 49 patients with NAFLD and DM; group II including 50 non-diabetic NAFLD patients; and the control including 52 normal subjects as group III | CIMT on carotid ultrasound | Ultrasound | There is a significant association between the presence of NAFLD and atherosclerosis, but this association was independent of DM. The grade of NAFLD and elevated liver function tests had no effect on severity of atherosclerosis |

NAFLD: Non-alcoholic fatty liver disease; CT: Computed tomography; CAC: Coronary artery calcification; CIMT: Carotid intima-media thickness; CP: Carotid plaque; DM: Diabetes mellitus.

Program (NCEP) requires the presence of 3 or more of the following components: (1) increased triglyceride levels (≥ 150 mg/dL); (2) low HDL level (< 40 mg/dL in men, < 50 mg/dL in women); (3) increased fasting glucose level (≥ 110 mg/dL); (4) hypertension (≥ 130/85 mm Hg or on antihypertensive medication); and (5) abdominal obesity (waist circumference: > 102 cm in men, > 88 cm in women).

Several different methods are used in the general population to estimate CVD risk including the Framingham Risk Score (FRS). The FRS is a validated measure of CV risk in the general population. The FRS predicts an individual's 10-year risk of myocardial infarction or CV death and incorporates age, sex, cholesterol, HDL, smoking status, and hypertension. Furthermore, the FRS has been validated as a predictor of CVD in NAFLD and should be used to risk-stratify individuals and guide treatment of risk factors including dyslipidemia. Recently, the American Heart Association recommended a new cardiovascular assessment tool for prediction of atherosclerotic cardiovascular disease. This score incorporates the usual risk factors for CVD but needs to be validated in patients with NAFLD[25].

## ASSESSMENT OF DYSLIPIDEMIA IN NAFLD

Dyslipidemia is frequent in individuals with NAFLD. The dyslipidemia in NAFLD is characterized by increased

serum triglycerides, increased small, dense low-density lipoprotein (LDL non-type A) particles, and low high-density lipoprotein (HDL) cholesterol. Recently, the value of non-HDL-C has been demonstrated in predicting coronary heart disease. Non-HDL-C is superior in predicting incidence of cardiovascular events and cardiac death in NAFLD patients compared to the traditional marker low-density lipoprotein[25]. The Adult Treatment Panel III of the National Cholesterol Education Program has added non-HDL-C to its recommended screening algorithm for assessing cardiovascular disease risk.

Non-HDL-C is a calculated value derived by subtracting HDL cholesterol from the total cholesterol (TC) level, both available on traditional lipid panels, and requires no additional cost. In addition, because it is derived from TC and HDL levels, which are not impacted by fasting, non-HDL-C does not require fasting for accuracy.

Because patients with NAFLD have a high prevalence of cardiovascular disease, the use of non-HDL-C provides an important value in cardiovascular risk stratification and as a target for lipid-lowering therapy.

Non-HDL-C levels are increased in patients with NASH compared with those with steatosis, particularly in those persons who are not receiving lipid-lowering medications.

## MANAGEMENT OF CARDIOVASCULAR DISEASE RISK IN NAFLD

All patients with NAFLD, irrespective of their body weight, should be advised lifestyle modifications in the form of regular exercise. Those who are overweight or obese are advised weight reduction. Regular exercise has been shown to improve the insulin sensitivity even without weight reduction. An exercise regimen should aim to achieve a target heart rate of 60%-70% of maximal heart rate through exercises such as brisk walking, jogging, or other aerobic exercises for at least 30 min, 5 d per week. Initial weight reduction in patients who are overweight or obese should be 10% of the body weight to be achieved in 6-8 mo. Overall, these overweight and obese patients need to create a negative balance by consuming fewer calories and burning more calories through regular exercise.

## TREATMENT OF DYSLIPIDEMIA

Lifestyle modification, encompassing weight loss and increased physical activity, is the cornerstone of dyslipidemia management in NAFLD. However, for groups with increased CVD risk, lifestyle modification should be accompanied by lipid-lowering therapy. Guidelines set forth by the NCEP Adult Treatment Panel Ⅲ provide guidance on which groups should be targeted for lipid-lowering therapy and outlines treatment goals[26]. These guidelines were not designed specifically to address dyslipidemia in individuals with NAFLD; however, they can be applied safely to individuals with NAFLD.

In this context, the most attractive group of lipid-lowering agents for cardiovascular protection are the statins. In addition to a major effect in lowering LDL, they have modest effects on increasing HDL and lowering serum triglycerides, as well as non- cholesterol-lowering effects on vascular endothelium by inducing endothelial nitric oxide synthase.

Statin hepatotoxicity has not been shown to be of increased risk in NAFLD. The Liver Expert Panel stated in a report in 2014 that statins can be safely used in NAFLD and NASH, and routine liver enzyme monitoring need not be done. Statins can be safely used in patients with decompensated liver disease. There is always concern for using high dose statins in patients with elevated liver enzymes; in such circumstances adding ezetimibe has a synergistic effect with statins[26,27].

Ezetimibe is less effective as a single agent to lower serum cholesterol and does not have the same vascular protective effects as statins. Ezetemibe is also useful when patients experience partially dose-dependent statin adverse effects such as myopathy.

Evidence that cholesterol lowering with statins reduces cardiovascular risk comes from the Greek Atorvastatin and Coronary Heart Disease Evaluation study, in which atorvastatin reduced the incidence of new cardiovascular events to a greater extent in patients with NAFLD (assumed by raised liver enzymes) than among those with normal liver enzymes[28]. Patients without serum cholesterol elevation benefited as well, and there was a 40% reduction in serum triglyceride.

Triglyceride elevations are also a risk factor for cardiovascular disease, albeit less so than cholesterol. Attempts to reduce raised serum triglyceride levels center around weight reduction, improving insulin resistance (physical activity) and diabetic control, with use of polyunsaturated fatty acids (fish oil) as the first-line pharmacologic approach. Ezetimibe, has been compared with placebo for the treatment of NASH in the MOZART randomized clinical trial. In secondary analysis of the MOZART trial, FRS and CAC score improved in a greater proportion of patients with ezetimibe but did not reach statistical significance[29].

## TREATMENT OF DIABETES MELLITUS

DM is associated with an increased risk of CVD. Because DM is highly prevalent among individuals with NAFLD, comprehensive management is essential for CVD risk reduction. A detailed discussion of the management of DM in individuals with NAFLD is beyond the scope of this review. However, primary and secondary prevention of CVD events in individuals with DM should focus on multifactorial risk reduction, including treatment of hypertension and dyslipidemia. In addition, specific treatments of DM, including metformin, may decrease CVD events.

## CONCLUSION

In the past several years, compelling evidence has

substantiated a strong link between NAFLD and increased risk of cardiovascular disease in individuals with or without coexisting metabolic syndrome. NAFLD is now recognized as a risk factor for poor cardiovascular outcomes including mortality and morbidity from major vascular events. As a whole, NAFLD patients may benefit from more careful surveillance and early treatment interventions. However, despite evidence linking increased cardiovascular risk with NAFLD, there is still uncertainty regarding the prognostic role of NAFLD in risk stratification for CHD. Additional, large follow-up studies are needed to establish whether adding NAFLD to the currently available risk scoring systems will improve cardiovascular disease risk prediction. Furthermore, the question of whether the prognostic value of NAFLD in the development and progression of cardiovascular disease only applies to NASH or also is associated with simple steatosis remains unresolved. Finally, more research is needed to understand the pathophysiology linking NAFLD with cardiovascular disease and to better elucidate whether genetic traits in NAFLD carry the same cardiovascular risk as metabolic syndrome-associate NAFLD.

## REFERENCES


1 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231 [PMID: 11961152 DOI: 10.1056/NEJMra011775]

2 **Kotronen A**, Yki-Järvinen H. Fatty liver: a novel component of the metabolic syndrome. *Arterioscler Thromb Vasc Biol* 2008; **28**: 27-38 [PMID: 17690317 DOI: 10.1161/ATVBAHA.107.147538]

3 **Dunn W**, Xu R, Wingard DL, Rogers C, Angulo P, Younossi ZM, Schwimmer JB. Suspected nonalcoholic fatty liver disease and mortality risk in a population-based cohort study. *Am J Gastroenterol* 2008; **103**: 2263-2271 [PMID: 18684196 DOI: 10.1111/j.1572-0241.2008.02034.x]

4 **Ruttmann E**, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H. Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality: an epidemiological investigation in a cohort of 163,944 Austrian adults. *Circulation* 2005; **112**: 2130-2137 [PMID: 16186419 DOI: 10.1161/CIRCULATIONAHA.105.552547]

5 **Wannamethee G**, Ebrahim S, Shaper AG. Gamma-glutamyltransferase: determinants and association with mortality from ischemic heart disease and all causes. *Am J Epidemiol* 1995; **142**: 699-708 [PMID: 7572939]

6 **Fraser A**, Harris R, Sattar N, Ebrahim S, Smith GD, Lawlor DA. Gamma-glutamyltransferase is associated with incident vascular events independently of alcohol intake: analysis of the British Women's Heart and Health Study and Meta-Analysis. *Arterioscler Thromb Vasc Biol* 2007; **27**: 2729-2735 [PMID: 17932318 DOI: 10.1161/ATVBAHA.107.152298]

7 **Hamaguchi M**, Kojima T, Takeda N, Nagata C, Takeda J, Sarui H, Kawahito Y, Yoshida N, Suetsugu A, Kato T, Okuda J, Ida K, Yoshikawa T. Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. *World J Gastroenterol* 2007; **13**: 1579-1584 [PMID: 17461452]

8 **Haring R**, Wallaschofski H, Nauck M, Dörr M, Baumeister SE, Völzke H. Ultrasonographic hepatic steatosis increases prediction of mortality risk from elevated serum gamma-glutamyl transpeptidase levels. *Hepatology* 2009; **50**: 1403-1411 [PMID: 19670414 DOI: 10.1002/hep.23135]

9 **Adams LA**, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005; **129**: 113-121 [PMID: 16012941]

10 **Arulanandan A**, Ang B, Bettencourt R, Hooker J, Behling C, Lin GY, Valasek MA, Ix JH, Schnabl B, Sirlin CB, Loomba R. Association Between Quantity of Liver Fat and Cardiovascular Risk in Patients With Nonalcoholic Fatty Liver Disease Independent of Nonalcoholic Steatohepatitis. *Clin Gastroenterol Hepatol* 2015; **13**: 1513-1520.e1 [PMID: 25661453 DOI: 10.1016/j.cgh.2015.01.027]

11 **Söderberg C**, Stål P, Askling J, Glaumann H, Lindberg G, Marmur J, Hultcrantz R. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. *Hepatology* 2010; **51**: 595-602 [PMID: 20014114 DOI: 10.1002/hep.23314]

12 **Zeb I**, Li D, Budoff MJ, Katz R, Lloyd-Jones D, Agatston A, Blumenthal RS, Blaha MJ, Blankstein R, Carr J, Nasir K. Nonalcoholic Fatty Liver Disease and Incident Cardiac Events: The Multi-Ethnic Study of Atherosclerosis. *J Am Coll Cardiol* 2016; **67**: 1965-1966 [PMID: 27102512 DOI: 10.1016/j.jacc.2016.01.070]

13 **Targher G**, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-alcoholic fatty liver disease: causal effect or epiphenomenon? *Diabetologia* 2008; **51**: 1947-1953 [PMID: 18762907 DOI: 10.1007/s00125-008-1135-4]

14 **Francque SM**, van der Graaff D, Kwanten WJ. Non-alcoholic fatty liver disease and cardiovascular risk: Pathophysiological mechanisms and implications. *J Hepatol* 2016; **65**: 425-443 [PMID: 27091791 DOI: 10.1016/j.jhep.2016.04.005]

15 **Kim D**, Choi SY, Park EH, Lee W, Kang JH, Kim W, Kim YJ, Yoon JH, Jeong SH, Lee DH, Lee HS, Larson J, Therneau TM, Kim WR. Nonalcoholic fatty liver disease is associated with coronary artery calcification. *Hepatology* 2012; **56**: 605-613 [PMID: 22271511 DOI: 10.1002/hep.25593]

16 **Sinn DH**, Cho SJ, Gu S, Seong D, Kang D, Kim H, Yi BK, Paik SW, Guallar E, Cho J, Gwak GY. Persistent Nonalcoholic Fatty Liver Disease Increases Risk for Carotid Atherosclerosis. *Gastroenterology* 2016; **151**: 481-488.e1 [PMID: 27283259 DOI: 10.1053/j.gastro.2016.06.001]

17 **Nahandi MZ**, Khoshbaten M, Ramazanzadeh E, Abbaszadeh L, Javadrashid R, Shirazi KM, Gholami N. Effect of non-alcoholic fatty liver disease on carotid artery intima-media thickness as a risk factor for atherosclerosis. *Gastroenterol Hepatol Bed Bench* 2014; **7**: 55-62 [PMID: 25436098]

18 **Marchesini G**, Moscatiello S, Di Domizio S, Forlani G. Obesity-associated liver disease. *J Clin Endocrinol Metab* 2008; **93**: S74-S80 [PMID: 18987273 DOI: 10.1210/jc.2008-1399]

19 **Villanova N**, Moscatiello S, Ramilli S, Bugianesi E, Magalotti D, Vanni E, Zoli M, Marchesini G. Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 473-480 [PMID: 15981216 DOI: 10.1002/hep.20781]

20 **Fracanzani AL**, Tiraboschi S, Pisano G, Consonni D, Baragetti A, Bertelli C, Norata D, Valenti L, Grigore L, Porzio M, Catapano A, Fargion S. Progression of carotid vascular damage and cardiovascular events in non-alcoholic fatty liver disease patients compared to the general population during 10 years of follow-up. *Atherosclerosis* 2016; **246**: 208-213 [PMID: 26803429 DOI: 10.1016/j.atherosclerosis.2016.01.016]

21 **Assy N**, Djibre A, Farah R, Grosovski M, Marmor A. Presence of coronary plaques in patients with nonalcoholic fatty liver disease. *Radiology* 2010; **254**: 393-400 [PMID: 20093511 DOI: 10.1148/radiol.09090769]

22 **Akabame S**, Hamaguchi M, Tomiyasu K, Tanaka M, Kobayashi-Takenaka Y, Nakano K, Oda Y, Yoshikawa T. Evaluation of vulnerable coronary plaques and non-alcoholic fatty liver disease (NAFLD) by 64-detector multislice computed tomography (MSCT). *Circ J* 2008; **72**: 618-625 [PMID: 18362435]

23 **Qasim A**, Mehta NN, Tadesse MG, Wolfe ML, Rhodes T, Girman C, Reilly MP. Adipokines, insulin resistance, and coronary artery calcification. *J Am Coll Cardiol* 2008; **52**: 231-236 [PMID: 18617073 DOI: 10.1016/j.jacc.2008.04.016]

24 **Oni ET**, Agatston AS, Blaha MJ, Fialkow J, Cury R, Sposito A, Erbel R, Blankstein R, Feldman T, Al-Mallah MH, Santos RD, Budoff MJ, Nasir K. A systematic review: burden and severity of subclinical cardiovascular disease among those with nonalcoholic

fatty liver; should we care? *Atherosclerosis* 2013; **230**: 258-267 [PMID: 24075754 DOI: 10.1016/j.atherosclerosis.2013.07.052]

25 **National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III).** Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; **106**: 3143-3421 [PMID: 12485966]

26 **Bays H**, Cohen DE, Chalasani N, Harrison SA. An assessment by the Statin Liver Safety Task Force: 2014 update. *J Clin Lipidol* 2014; **8**: S47-S57 [PMID: 24793441 DOI: 10.1016/j.jacl.2014.02.011]

27 **Averna M**. The effect of ezetimibe on NAFLD. *Atheroscler Suppl* 2015; **17**: 27-34 [PMID: 25659874 DOI: 10.1016/S1567-5688(15)50007-X]

28 **Athyros VG**, Tziomalos K, Gossios TD, Griva T, Anagnostis P, Kargiotis K, Pagourelias ED, Theocharidou E, Karagiannis A, Mikhailidis DP. Safety and efficacy of long-term statin treatment for cardiovascular events in patients with coronary heart disease and abnormal liver tests in the Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) Study: a post-hoc analysis. *Lancet* 2010; **376**: 1916-1922 [PMID: 21109302 DOI: 10.1016/S0140-6736(10)61272-X]

29 **Lin SC**, Ang B, Hernandez C, Bettencourt R, Jain R, Salotti J, Richards L, Kono Y, Bhatt A, Aryafar H, Lin GY, Valasek MA, Sirlin CB, Brouha S, Loomba R. Cardiovascular risk assessment in the treatment of nonalcoholic steatohepatitis: a secondary analysis of the MOZART trial. *Therap Adv Gastroenterol* 2016; **9**: 152-161 [PMID: 26929777 DOI: 10.1177/1756283X15621232]

30 **Ekstedt M**, Hagström H, Nasr P, Fredrikson M, Stål P, Kechagias S, Hultcrantz R. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* 2015; **61**: 1547-1554 [PMID: 25125077 DOI: 10.1002/hep.27368]

31 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]

32 **Pickhardt PJ**, Hahn L, Muñoz del Rio A, Park SH, Reeder SB, Said A. Natural history of hepatic steatosis: observed outcomes for subsequent liver and cardiovascular complications. *AJR Am J Roentgenol* 2014; **202**: 752-758 [PMID: 24660702 DOI: 10.2214/AJR.13.11367]

33 **Kim D**, Kim WR, Kim HJ, Therneau TM. Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. *Hepatology* 2013; **57**: 1357-1365 [PMID: 23175136 DOI: 10.1002/hep.26156]

34 **Emre A**, Terzi S, Celiker E, Sahin S, Yazıcı S, Erdem A, Ceylan US, Asik M, Yesilcimen K. Impact of Nonalcoholic Fatty Liver Disease on Myocardial Perfusion in Nondiabetic Patients Undergoing Primary Percutaneous Coronary Intervention for ST-Segment Elevation Myocardial Infarction. *Am J Cardiol* 2015; **116**: 1810-1814 [PMID: 26506122 DOI: 10.1016/j.amjcard.2015.09.021]

35 **Pais R**, Giral P, Khan JF, Rosenbaum D, Housset C, Poynard T, Ratziu V. Fatty liver is an independent predictor of early carotid atherosclerosis. *J Hepatol* 2016; **65**: 95-102 [PMID: 27129836 DOI: 10.1016/j.jhep.2016.02.023]

36 **Park HE**, Kwak MS, Kim D, Kim MK, Cha MJ, Choi SY. Nonalcoholic Fatty Liver Disease Is Associated With Coronary Artery Calcification Development: A Longitudinal Study. *J Clin Endocrinol Metab* 2016; **101**: 3134-3143 [PMID: 27253666 DOI: 10.1210/jc.2016-1525]

**P- Reviewer**: Ding WJ, Zhu X **S- Editor**: Qi Y **L- Editor**: A
**E- Editor**: Wu HL

World Journal of Gastrointestinal Pathophysiology

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

*World J Gastrointest Pathophysiol* 2017 May 15; 8(2): 59-66

DOI: 10.4291/wjgp.v8.i2.59

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Basic Study

# Rectification of oxygen transfer through the rat colonic epithelium

Fernando D Saraví, Graciela E Carra, Daniel A Matus, Jorge E Ibáñez

Fernando D Saraví, Graciela E Carra, Jorge E Ibáñez, Institute of Physiology, Faculty of Medical Sciences, National University of Cuyo, Mendoza 5500, Argentina

Daniel A Matus, Department of Surgery, Faculty of Medical Sciences, National University of Cuyo, Mendoza 5500, Argentina

Jorge E Ibáñez, Mario H. Burgos Institute of Histology and Embryology, CONICET, Faculty of Medical Sciences, National University of Cuyo, Mendoza 5500, Argentina

Author contributions: Saraví FD and Carra GE designed study concept and supervised the experiments; Matus DA and Ibáñez JE performed the experiments and analyzed the data; all authors contributed to data interpretation; Saraví FD drafted the manuscript, which was then critically revised by Carra GE, Matus DA and Ibáñez JE.

Supported by The Secretaría de Ciencia, Técnica y Posgrado, National University of Cuyo, Mendoza 5500 Argentina to Saraví FD, No. 06/J457.

Institutional review board statement: This study was reviewed and approved by the Secretaría de Ciencia y Técnica, National University of Cuyo, institutional review board.

Institutional animal care and use committee statement: All procedures involving the care and use of animals were approved by the Committee for Animal Care and Use of the Faculty of Medical Sciences, National University of Cuyo, Mendoza 5500, Argentina.

Conflict-of-interest statement: There is no conflict of interest.

Data sharing statement: There are no additional data available.



Manuscript source: Invited manuscript

Correspondence to: Fernando D Saraví, MD, PhD, Full Professor, Institute of Physiology, Faculty of Medical Sciences, National University of Cuyo, Parque General San Martín s/n, Mendoza 5500, Argentina. fernando.saravi@hotmail.es
Telephone: +54-261-4135000-2756
Fax: +54-261-4203288

Received: January 26, 2017
Peer-review started: February 8, 2017
First decision: March 9, 2017
Revised: April 12, 2017
Accepted: April 23, 2017
Article in press: April 24, 2017
Published online: May 15, 2017

## Abstract

***AIM***

To assess whether higher sensitivity of colonic epithelium to hypoxia at the serosal side is associated with oxygen transfer asymmetry.

***METHODS***

Rats were fed either with normal chow or a low-sodium diet. Tissues were mounted as flat sheets in a modified, airtight Ussing chamber with oxygen meters in each hemichamber. Mucosal samples from normal diet animals were studied under control conditions, in low-chloride solution and after adding chloride secretion inhibitors and chloride secretagogues. Samples from sodium-deprived rats were studied before and after ouabain addition. In separate experiments, the correlation between short-circuit current and oxygen consumption was analyzed. Finally, hypoxia was induced in one hemichamber to assess the relationship between its oxygen content and the oxygen pressure difference

between both hemichambers.

***RESULTS***

In all studied conditions, oxygen consumption was larger in the serosal hemichamber than in the mucosal one ($P$ = 0.0025 to $P$ < 0.0001). Short-circuit current showed significant correlation with both total oxygen consumption ($r$ = 0.765; $P$ = 0.009) in normoxia and oxygen consumption in the serosal hemichamber ($r$ = 0.754; $P$ = 0.011) during mucosal hypoxia, but not with oxygen consumption in the mucosal hemichamber. When hypoxia was induced in the mucosal hemichamber, an oxygen pressure difference of 13 kPa with the serosal hemichamber was enough to keep its oxygen content constant. However, when hypoxia was induced in the serosal hemichamber, the oxygen pressure difference with the mucosal hemichamber necessary to keep its oxygen content constant was 40 kPa ($P$ < 0.0001).

***CONCLUSION***

Serosal oxygen supply is more readily available to support short-circuit current. This may be partly due to a rectifying behavior of transepithelial oxygen transfer.

**Key words:** Colonic epithelium; Hypoxia; Oxygen diffusion; Short-circuit current; Ussing chamber



**Core tip:** The physiological dependence of the colonic epithelium on oxygen provided from the serosal side is not only due to the structure of its blood supply and the low oxygen pressure of colonic intraluminal contents, since it is also observed in isolated mucosa preparations. This study demonstrates for the first time that a much larger partial pressure difference is needed for oxygen transfer from the mucosal side to the serosal side of the epithelium than for transfer in the opposite direction, a phenomenon that may be considered a rectifying behavior.

Saraví FD, Carra GE, Matus DA, Ibáñez JE. Rectification of oxygen transfer through the rat colonic epithelium. *World J Gastrointest Pathophysiol* 2017; 8(2): 59-66 Available from: URL: http://www.wjgnet.com/2150-5330/full/v8/i2/59.htm DOI: http://dx.doi.org/10.4291/wjgp.v8.i2.59

## INTRODUCTION

Hypoxia is considered the earliest factor causing organ damage in intestinal ischemia[1], caused, for example, by hypoperfusion associated with septic shock[2], general anesthesia[3], or ischemic colitis; the latter condition is the most frequent form of ischemic injury to the gastrointestinal tract[4,5].

The intestinal epithelium is very sensitive to hypoperfusion. Epithelial ion transport is closely coupled to aerobic metabolism[6,7]. However, the intestinal epithelium normally has relatively low oxygen partial pressure ($P_{O2}$), a condition characterized as "physiologic hypoxia"[8]. Hypothetically, oxygen might be provided to the epithelium from both the luminal and the serosal side. However, the composition of intraluminal gas is variable and, as a rule, its $P_{O2}$ is lower than that of either arterial or venous blood[9,10].

While oxygen consumption by intraluminal bacteria may partially account for the differences, it has been reported that intracolonic $P_{O2}$ was less than 2 mmHg higher in germ-free rats than in control rats[11]. Luminal oxygen is an unlikely source for epithelial consumption, since intraluminal $P_{O2}$ is lower than mucosal $P_{O2}$[12]. In the intact colon *in vivo*, oxygen reaches the epithelial cells through their basolateral membranes by diffusion from the capillary network which surrounds the crypts[8,13].

In most studies of epithelial biology employing Ussing chambers, both sides of the samples are oxygenated. If unremoved, in the intestinal epithelium both the adherent mucus gel layer and the submucosal tissue restrain oxygen diffusion[14]. Hypoxia induced in the serosal hemichamber while keeping a high oxygen pressure at the mucosal side lowers short-circuit current ($I_{sc}$) just as effectively and with the same time-course as hypoxia simultaneously induced in both sides. On the other hand, hypoxia induced in the mucosal hemichamber does not reduce $I_{sc}$ as long as the serosal side remained oxygenated[15]. Furthermore, non-everted colonic sacs oxygenated from the serosal side showed a 127% higher $I_{sc}$ than everted sacs oxygenated from the mucosal side[15]. One possible cause of the observed asymmetry is higher serosal than mucosal oxygen consumption ($QO_2$); another is some kind of barrier, present at the mucosal but not at the serosal side, which hinders oxygen diffusion.

Our working hypotheses were, first, that the observed asymmetries are due to an intrinsic property of the epithelium, and therefore they should be found under different experimental treatments. Second, that the epithelial hindrance to oxygen diffusion may be different for transfer from lumen to interstitium than in the reverse direction. This may be deemed a rectifying behavior by analogy to electrical devices. Rectification is a property of diodes, which are electrical devices that allow current flow in one direction far more easily than in the other. Some membrane ion channels display rectifying behavior, since they show a nonlinear relationship between the driving force (potential difference) and the resulting current[16].

The aims of this paper are, first, to assess the differences in oxygen consumption provided from the apical side of the epithelium and from its serosal side during different experimental treatments and, second, to measure the partial pressure difference needed for oxygen transfer from the mucosal to the serosal side and for oxygen transfer in the opposite direction.

## MATERIALS AND METHODS

### *Animals*

Wistar-Hokkaido male rats weighing 250-300 g were housed and managed according to the guidelines for animal care and biosafety of our Medical School. The animals were kept at an environmental temperature of 25 ℃ ± 1 ℃ with a 12-h light-dark cycle.

All animals were given food and drink *ad libitum*. A group of rats drank tap water and ate a standard rat chow containing 1.77 mg of sodium per gram of food (76.9 mEq/kg; Cargill Co.). A second group was given distilled water and a low-sodium diet with 0.161 mg of sodium per gram of food (7 mEq/kg; ICN Flow Catalog # 902902) for 10 d. The low-sodium diet was purchased from ICN, Inc. (Costa Mesa, California, United States). Daily food consumption was recorded in both groups.

All procedures involving the care and use of animals were approved by the Committee for Animal Care and Use of the Faculty of Medical Sciences, National University of Cuyo. This study was reviewed and approved by the Secretaría de Ciencia y Técnica, National University of Cuyo, institutional review board.

### *Serum aldosterone determination*

Blood samples from all rats fed with the sodium-deficient diet and an equal number of rats fed with standard chow were obtained during the surgical procedure. Blood was allowed to clot; serum was extracted and frozen at -70 ℃. Aldosterone concentration in stored sera was afterwards measured with a coated-tube radioimmunoassay (Diagnostic Products Corporation, Los Angeles, California, United States).

### *Dissection and mounting*

Under ether anesthesia, the entire colon was excised. A 3-cm segment was cut from the descending portion just above the pelvic brim and dissected to obtain an isolated mucosa preparation, as previously described[15]. The segment was cut open along its mesenteric border, the adherent mucus gel layer was gently removed with a cotton tip soaked in dissecting solution and the segment was mounted as a flat sheet in a modified Ussing chamber (described below).

### *Solutions and gas mixtures*

The composition of the standard Ringer solution employed was as follows (in mmol/L): Na 132.8; K 4.5; Ca 1.25; Mg 1.0; Cl 114.0; $HCO_3$ 24.0; $H_2PO_4$ 1.0; $SO_4$ 1.0; D (+) glucose 10.0. In the low-chloride solution, most chloride was replaced by sulfate, with the addition of mannitol to compensate for the difference in osmolality[11], as follows (in mmol/L): Na 136.6; K 4.5; Ca 1.25: Mg 1.0; Cl 2.5; $HCO_3$ 24.0; $H_2PO_4$ 2.0; $SO_4$ 58.1; D (+) glucose 10.0; mannitol 93.4. The osmolality 0f both solutions was 280 mOsm/kg $H_2O$.

Two gas mixtures were used: 950 mL/L $O_2$ - 50 mL/L $CO_2$ (normoxic mixture) and 950 mL/L $N_2$ - 50 mL/L $CO_2$ (hypoxic mixture). The gas mixtures were purchased from Air Liquide, Inc. (Buenos Aires, Argentina). Their compositions were certified by the company. When gassed with either gas mixture, the pH of both solutions was 7.40.

### *Drugs*

Bumetanide, 3-methyl-1-isobutilxanthine (IBMX), serotonin, and amiloride were purchased from Sigma-Aldrich (St Louis, Missouri, United States), and diphenylamine-2-carboxylate (DPC) from ICN, Inc (Costa Mesa, California, United States). The appropriate drug solutions were prepared just before each experiment. The standard Ringer solution was employed to dissolve serotonin and carbachol; dimethylsulfoxide was used for IBMX and bumetanide, and absolute ethanol was used for amiloride and DPC. As indicated, bumetanide, IBMX and serotonin were added to the serosal hemichamber, while DPC and amiloride were added to the mucosal hemichamber to achieve the following concentrations (in mmol/L): bumetanide 0.1; DPC 0.5; IBMX 0.1; serotonin 0.1, and amiloride 0.1. At the start of each experiment, 91 μg/mL gentamycin (Schering-Plough, Buenos Aires, Argentina) was added to each hemichamber, to prevent bacterial overgrowth.

### *Oxymetric Ussing chamber and electrophysiological measurements*

For this report, a modified Ussing chamber which has already been described and validated was used[17]. The chamber is airtight and it has a 1 $cm^2$ window between the hemichambers. Polarimetric oxygen meters (CellOx 325, WTW GmbH, Weilheilm, Germany) are attached to each hemichamber. This arrangement allows measurement of the oxygen concentration and its time course in both the hemichamber facing the basolateral (serosal) side of the epithelium and the hemichamber facing its apical (mucosal) side. The chamber content was kept at 37 ℃ with a water jacket fed from a thermostatic reservoir.

The transepithelial potential difference was recorded with calomel electrodes connected to each hemichamber through saline bridges (agar-in-Ringer, 30 g/L). Ag-AgCl electrodes were used to supply current to the chamber from an amplifier, in order to clamp the transepithelial potential difference (TPD) at 0 mV. The output of the amplifier took into account corrections for solution resistance and bridge asymmetry. The short-circuit condition was kept throughout all experiments, except for the brief periods of release needed to measure open circuit TPD. The transepithelial resistivity was calculated, according to Ohm's law, as the quotient between TPD and $I_{sc}$.

### *Experimental procedures*

Before each experiment, oxygen probes were calibrated according to the user's manual and had their

slopes checked and recorded. At the beginning of each experiment, the hemichambers were gassed with the normoxic mixture until a plateau of their oxygen concentration was reached. Afterwards both hemichambers were hermetically closed. $QO_2$ was measured under baseline conditions for 30-min periods, starting 60 min after the closure of the hemichambers.

Solution replacement was carried out without opening the chamber with a gravity driven system which allowed passing from a water-jacketed reservoir (37 ℃) through the chamber a volume of low-chloride solution 10-fold larger than the chamber volume, with the overflow being drained through siphons. In experiments involving replacement of Ringer with low-chloride solution or addition of chloride secretion blockers, Isc was allowed to stabilize during 20 min before $QO_2$ was measured for the next 30 min. On the other hand, the 30-min $QO_2$ measurement was started immediately after addition of IBMX or serotonin.

The change in oxygen concentration in each hemichamber during the measurement period was used to calculate $QO_2$, as detailed elsewhere[11]. In experiments assessing oxygen transfer between the hemichambers, a baseline $QO_2$ was obtained after attaining the same oxygen level in both hemichambers. Then hypoxia was induced in one hemichamber by gassing its contents with 950 mL/L $N_2$ - 50 mL/L $CO_2$ for various times while bubbling 950 mL/L $O_2$ - 50 mL/L $CO_2$ in the other hemichamber, to obtain graded differences in oxygen pressure ($\Delta P_{O2}$) between the hemichambers. Afterwards the chamber was closed and the rate of change in oxygen concentration ($\Delta CO_2$) in the hypoxic hemichamber was plotted against the $\Delta P_{O2}$ between both sides.

After a 15-min period of reoxygenation of both hemichambers, the procedure described above was repeated for inducing hypoxia in the other hemichamber. The $\Delta P_{O2}$ was calculated as the difference in mean $P_{O2}$ of each hemichamber during each hypoxic period. The order in which hypoxia was induced in each hemichamber was switched between experiments to avoid a possible effect derived from the hemichamber which was made hypoxic first. Finally, both hemichambers were reoxygenated and a second control $QO_2$ measurement was performed.

After each experiment, the mucosa was replaced with a polyethylene membrane for a blankrun. The experiment was discarded if the decrease in oxygen concentration was above 5% of that observed with the biological sample.

### *Statistical analysis*

$QO_2$ in the serosal and mucosal hemichambers and $I_{sc}$ under each tested condition were compared with a paired, two-sided Student's *t* test. An unpaired, two-sided Student's *t* test was employed for analysis of food intake, sodium intake, and serum aldosterone in rats fed with normal sodium diet and in those submitted to the low-sodium diet. During analysis, significant deviations from a Gaussian distribution were ruled out with the Kolmogorov-Smirnov test.

A one-way analysis of variance with Geisser-Greenhouse correction was used to assess differences in the magnitude of the serosal *vs* mucosal difference in $QO_2$ under all conditions tested. Simple linear regression was used to assess the relationships between $I_{sc}$ and $\Delta P_{O2}$, and between $\Delta P_{O2}$ and $\Delta CO_2$. Checks for significant deviation from linearity and outliers were performed for all comparisons.

A commercial software was employed for statistical analyses (GraphPad Prism version 5.1 for Windows, GraphPad Software, San Diego, California, Δ). Values are reported as means ± SEM, unless otherwise stated. The significance level was set at $P < 0.05$.

## RESULTS

Mean daily food intake of rats fed with the low-sodium diet (80.0 ± 1.4 g per kilogram body weight; $n = 6$) was not significantly different from that of rats given standard chow (82.1 ± 0.9 g per kilogram body weight; $n = 20$). The respective mean daily sodium intakes per kilogram body weight were 12.9 ± 0.3 mg and 145.3 ± 1.6 mg ($P < 0.0001$). As expected, serum aldosterone concentration was significantly higher in rats with low sodium intake (10.49 ± 2.1 nmol/L; $n = 6$) than in controls (1.42 ± 0.26 nmol/L; $n = 6$, $P = 0.0016$).

Values of $I_{sc}$, total $QO_2$, serosal $QO_2$, and mucosal $QO_2$ for epithelial samples are shown in Table 1. Compared with controls, $I_{sc}$ and $QO_2$ were higher in the presence of chloride secretagogues and in epithelial samples from sodium-deprived rats. Conversely, $I_{sc}$ and $QO_2$ were lower in low-chloride solution, in the presence of chloride secretion blockers or, in tissues from sodium-deprived animals, after addition of amiloride. Under all conditions tested, serosal oxygen consumption was higher than mucosal oxygen consumption (Figure 1). The magnitude of this difference was similar for all treatments ($P = 0.0847$ according to one-way analysis of variance).

$I_{sc}$ was correlated with total $QO_2$ and serosal $QO_2$ both under baseline condition and during mucosal hypoxia (Figure 2), but those correlations were lost during serosal hypoxia. On the other hand, $I_{sc}$ was not correlated with mucosal $QO_2$ under any condition (Table 2).

When different oxygen tension differences were imposed between both hemichambers, the oxygen content of the hypoxic hemichamber increased with time when $\Delta P_{O2}$ was high but decreased when it was low (Figure 3). The relationship was linear when hypoxia was induced in either hemichamber. The slope for hypoxia induced in the mucosal hemichamber was 0.0535 ± 0.009 $\mu mol \times cm^{-2} \times h^{-1}$ per kPa. The slope for hypoxia induced in the serosal hemichamber was 0.0494 ± 0.014 $\mu mol \times cm^{-2} \times h^{-1}$ per kPa ($P = 0.8244$ for the comparison of both slopes). However, the calculated $\Delta P_{O2}$ at which

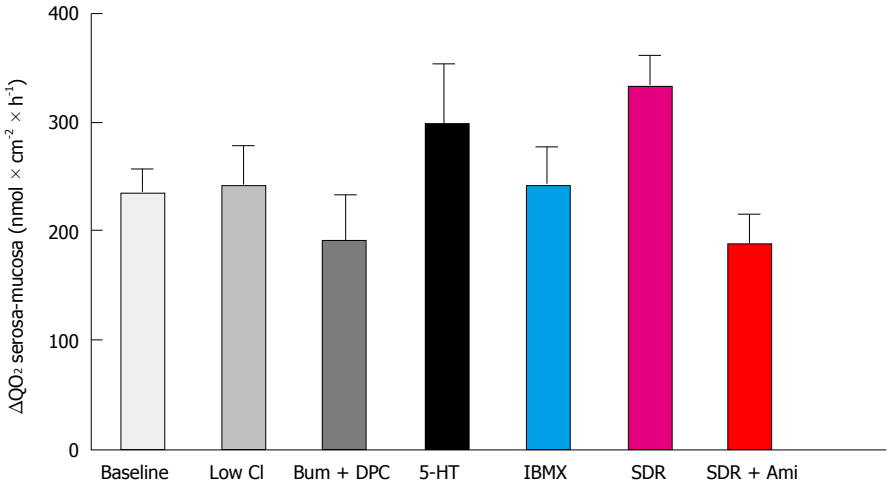


**Figure 1 Differences in serosal *vs* mucosal oxygen consumption of rat sigmoid colon epithelium under several conditions.** No significant difference between groups was found, as assessed by one-way analysis of variance ($P$ = 0.0849). $\Delta QO_2$: Serosa-mucosa, difference between serosal and mucosal oxygen consumption; Bum: Bumetanide; DPC: Diphenylamine-2-carboxylate; IBMX: 3-methyl-1-isobutilxanthine; SDR: Sodium-deprived rats; Ami: Amiloride.

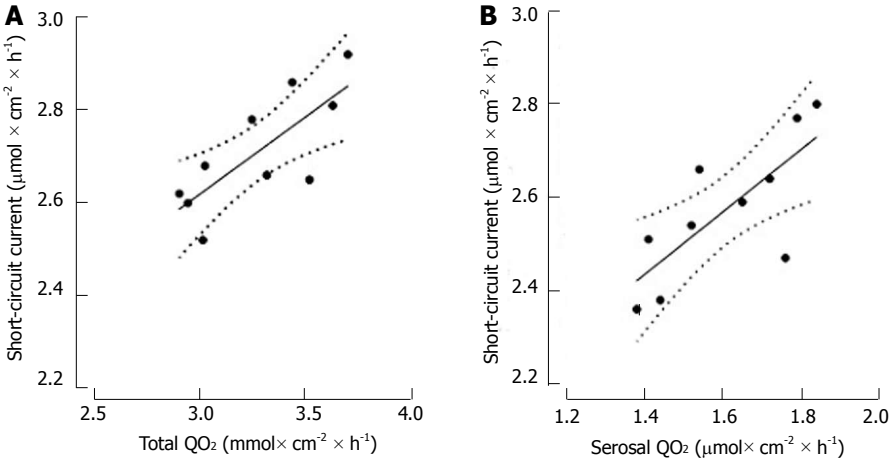


**Figure 2 Linear regression of short-circuit current *vs* total oxygen consumption during normoxia (A), and *vs* serosal oxygen consumption during mucosal hypoxia (B).** Regression coefficients were 0.765 for A ($P$ = 0.009) and 0.754 for B ($P$ = 0.011). No outliers were detected.

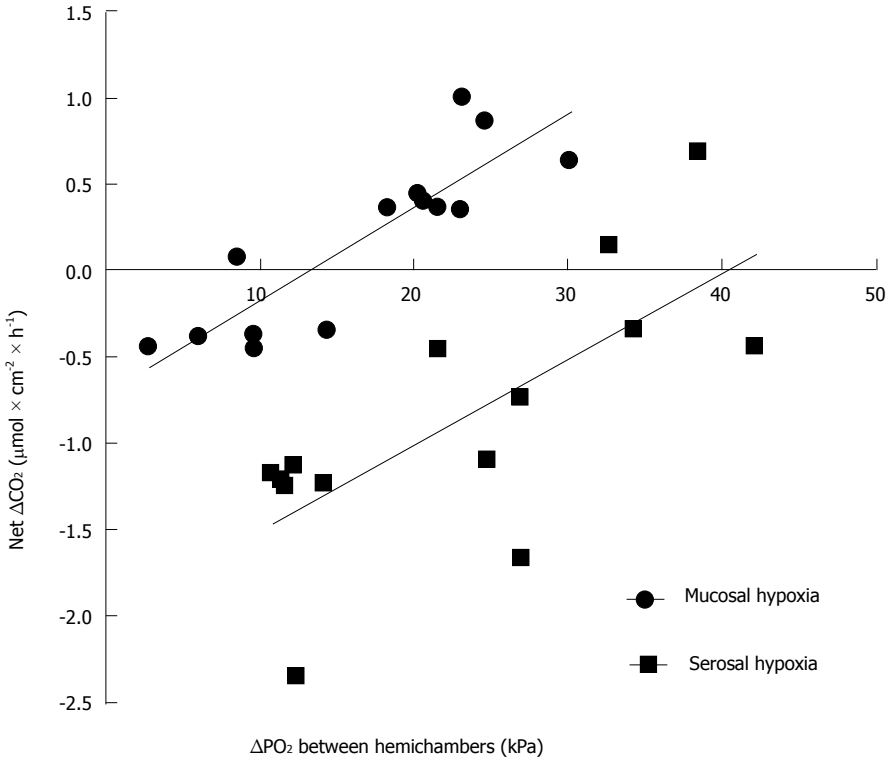


**Figure 3 Rate of change in oxygen content of the hypoxic hemichamber as a function of the difference in oxygen partial pressure between hemichambers.** Various degrees of hypoxia were induced in either the serosal or the mucosal hemichamber while keeping the opposite hemichamber fully oxigenated, and the change in oxygen content was plotted as a function of the mean difference in oxygen pressure between both hemichambers during a 30-min observation period. The slope of the relationships was the same when either the serosal or the mucosal hemichamber was hypoxic ($P$ = 0.8244), but the oxygen pressure difference at which there was no net change in oxygen content in the hypoxic hemichamber was larger when hypoxia was induced in the serosal hemichamber ($P$ < 0.0001). $\Delta CO_2$: Change in oxygen content of the hypoxic hemichamber; $\Delta PO_2$: Oxygen pressure difference between hemichambers.

**Table 1 Short-circuit current and oxygen consumption of the isolated mucosa of rat sigmoid colon**

| Treatment | *n* | Isc<br>μmol × cm$^{-2}$ × h$^{-1}$ | Total $QO_2$<br>μmol × cm$^{-2}$ × h$^{-1}$ | Serosal $QO_2$<br>μmol × cm$^{-2}$ × h$^{-1}$ | Mucosal $QO_2$<br>μmol × cm$^{-2}$ × h$^{-1}$ |
|---|---|---|---|---|---|
| None | 20 | 3.33 ± 0.21 | 2.79 ± 0.06 | 1.52 ± 0.04 | 1.28 ± 0.08[b] |
| Low Cl | 6 | 0.66 ± 0.07 | 2.56 ± 0.13 | 1.41 ± 0.08 | 1.14 ± 0.06[d] |
| Bum + DPC | 6 | 0.63 ± 0.08 | 2.47 ± 0.15 | 1.35 ± 0.07 | 1.15 ± 0.08[d] |
| Serotonin | 6 | 5.30 ± 0.29 | 3.64 ± 0.13 | 1.97 ± 0.08 | 1.67 ± 0.06[d] |
| IBMX | 6 | 3.98 ± 0.24 | 3.22 ± 0.09 | 1.75 ± 0.07 | 1.47 ± 0.06[d] |
| SDR | 6 | 8.50 ± 0.45 | 3.71 ± 0.35 | 2.02 ± 0.15 | 1.68 ± 0.12[b] |
| SDR + Ami | 6 | 0.33 ± 0.05 | 2.88 ± 0.12 | 1.53 ± 0.07 | 1.35 ± 0.08[d] |

[b]$P < 0.0001$ and [d]$P < 0.01$ for the difference between serosal and mucosal oxygen consumption. Isc: Short-circuit current; $QO_2$: Oxygen consumption; Bum: Bumetanide; DPC: Diphenylamine-2-carboxylate; IBMX: 3-methyl-1-isobutilxanthine; SDR: Sodium-deprived rats; Ami: Amiloride.

**Table 2 Relationship between short-circuit current and epithelial oxygen consumption of the isolated mucosa of rat sigmoid colon**

| Condition | Comparison | $R^2$ |
|---|---|---|
| Baseline | $I_{sc}$ *vs* total $QO_2$ | 0.586[b] |
| | $I_{sc}$ *vs* serosal $QO_2$ | 0.620[c] |
| | $I_{sc}$ *vs* mucosal $QO_2$ | 0.299 |
| Mucosal hypoxia | $I_{sc}$ *vs* total $QO_2$ | 0.633[d] |
| | $I_{sc}$ *vs* serosal $QO_2$ | 0.569[g] |
| | $I_{sc}$ *vs* mucosal $QO_2$ | 0.388 |
| Serosal hypoxia | $I_{sc}$ *vs* total $QO_2$ | 0.164 |
| | $I_{sc}$ *vs* serosal $QO_2$ | 0.315 |
| | $I_{sc}$ *vs* mucosal $QO_2$ | 0.361 |

[b]$P = 0.009$; [c]$P = 0.018$; [d]$P = 0.006$; [g]$P = 0.011$; $n = 10$. $I_{sc}$: Short-circuit current; $QO_2$: Oxygen consumption.

there was no change in oxygen content of the hypoxic hemichamber ($\Delta CO_2 = 0$) was three times higher for serosal hypoxia ($\Delta P_{O2} = 40$ kPa) than for mucosal hypoxia ($\Delta P_{O2} = 13$ kPa): $P < 0.0001$.

## DISCUSSION

Present results show that, when oxygen is available from both the serosal and the mucosal sides to distal colonic epithelium, the tissue consumes more oxygen supplied from the serosal than from the mucosal side. Since blood supply plays no role in oxygen availability under present *in vitro* conditions, the observed preference may not be explained by the path through which oxygen is normally provided to the epithelium. Furthermore, the higher serosal $QO_2$ was observed under a variety of conditions, including those in which chloride secretion is the major electrogenic phenomenon and those in which the major electrogenic phenomenon is sodium absorption. This suggests that the asymmetry is also not due to the specific ion being transported but represents a physiological characteristic of the colonic epithelium. It should be noted that this asymmetry has also been demonstrated in epithelial samples from human sigmoid colon[18].

The importance of oxygen supply from the serosal side to sustain electrogenic transport was further corroborated by the correlation between $I_{sc}$ and serosal $QO_2$ under baseline condition and during hypoxia induced in the mucosal hemichamber, while no such correlation was present during hypoxia induced in the serosal hemichamber. On the other hand, no correlation was found between $I_{sc}$ and mucosal $QO_2$ in any condition tested.

When graded hypoxia is induced in one hemichamber, while the other is saturated with oxygen, theoretically the oxygen content of the hypoxic hemichamber could decrease, remain constant, or increase with time. It would decrease if the hypoxic chamber still provides part of the oxygen consumed by the epithelium. It would remain constant if all oxygen consumed by the epithelium is provided by the oxygenated chamber and there is no net oxygen transfer between the hemichambers. Finally, it would actually increase if the oxygenated hemichamber, apart from providing all the oxygen that the epithelium consumes, transfers part of its oxygen to the hypoxic hemichamber. The relationship between $\Delta CO_2$ in the hypoxic hemichamber and $\Delta P_{O2}$ between the hemichambers was linear and showed the same slope for serosal-to-mucosal transfer than for mucosal-to serosal transfer. Remarkably, however, the $\Delta P_{O2}$ at which there was no net oxygen transfer between the hemichambers was three-fold higher for mucosal-to-serosal transfer than for transfer in the reverse direction, indicating that a larger $\Delta P_{O2}$ between hemichambers is needed for mucosal-to-serosal transfer than for serosal-to-mucosal transfer. This may be characterized as a rectifying behavior of oxygen transfer.

One explanation for the larger contribution of oxygen supply from the serosal side may be that the oxygen permeability of the basolateral membrane to oxygen is larger than the oxygen permeability of the apical membrane. While it is classically accepted that biological membranes are very permeable to gases, low permeability of some membranes to gases such as ammonia or carbon dioxide has been reported, for example in gastric glands[19] and colonic epithelium[20]. This issue has been recently reviewed, and the existence of gas channels has been postulated[21,22]. There are few reports on membrane oxygen permeability[21] and the results are contradictory[22].

Even if apical and basolateral epithelial membranes

had different oxygen permeability, this would not explain *per se* the apparent rectifying behavior of oxygen transfer. We are not aware of reports on this topic for oxygen, or for any other gas. Since gases generated in the colon as products of bacterial fermentation are partly transferred into the bloodstream[10], it is of physiological and clinical interest to assess whether their rate of transfer from the lumen to the interstitium is selectively limited, as it seems to be the case for oxygen.

In conclusion, the sigmoid colon epithelium *in vitro* in a rat model preferentially consumes oxygen supplied from the serosal side. $I_{sc}$ correlates with serosal oxygen consumption, but not mucosal oxygen consumption. The $\Delta P_{O2}$ for inducing net mucosal-to-serosal transfer is higher than the $\Delta P_{O2}$ for inducing serosal-to mucosal transfer, therefore indicating a rectifying behavior for transepithelial oxygen transfer.

## COMMENTS

### Background

The colonic epithelium has a high oxygen consumption, a large part of which is needed to sustain electrogenic ion transport. *In vivo*, oxygen is mostly supplied from the serosal side, since the luminal environment has a very low oxygen partial pressure. When the epithelium is placed in an Ussing chamber, oxygen is usually available from both sides of the epithelium, but even then, the serosal supply is more important to sustain electrogenic ion transport than the mucosal supply.

### Research frontiers

The intestinal epithelium is normally submitted to a relatively low oxygen pressure when compared with other tissues, a condition known as physiological hypoxia. This makes the epithelium susceptible to hypoxic injury when the oxygen supply is further compromised by disease.

### Innovations and breakthroughs

In this paper, it is shown that serosal supply provides more oxygen than mucosal supply to the colonic epithelium under several different conditions, suggesting that the difference is an intrinsic property of the tissue. For the first time, evidence is provided suggesting that transepithelial oxygen diffusion from serosa to mucosa needs a lower gradient of oxygen pressure than diffusion from mucosa to serosa.

### Applications

Since epithelial hypoxia seems to be an important cause of injury and dysfunction in several conditions affecting the intestinal epithelium, a deeper knowledge of the factors influencing epithelial oxygen supply may help to understand their pathophysiology and to devise better management strategies.

### Terminology

Rectification: A term borrowed from electronics, referring to devices such as diodes, which allow electric current to flow more easily in one direction than in the opposite one. In the present context, it is applied to the observation that oxygen diffuses more easily from the serosal to the mucosal side of the epithelium than in the opposite direction. Short-circuit current: The electrical current passing through the epithelium needed to keep the transepithelial potential difference at 0 mV. It is a measure of electrogenic ion transport.

### Peer-review

The aim of this study was to assess whether higher sensitivity of colonic epithelium to hypoxia at the serosal side is associated with oxygen transfer asymmetry. It is a very well design study and the issue is elegantly developed.

## REFERENCES


1 **MacDonald PH**. Ischaemic colitis. *Best Pract Res Clin Gastroenterol* 2002; **16**: 51-61 [PMID: 11977928 DOI: 10.1053/bega/2001.0265]
2 **van Haren FM**, Sleigh JW, Pickkers P, Van der Hoeven JG. Gastrointestinal perfusion in septic shock. *Anaesth Intensive Care* 2007; **35**: 679-694 [PMID: 17933153]
3 **Leung FW**. Endoscopic reflectance spectrophotometry and visible light spectroscopy in clinical gastrointestinal studies. *Dig Dis Sci* 2008; **53**: 1669-1677 [PMID: 17932761 DOI: 10.1007/s10620-007-0026-8]
4 **Theodoropoulou A**, Koutroubakis IE. Ischemic colitis: clinical practice in diagnosis and treatment. *World J Gastroenterol* 2008; **14**: 7302-7308 [PMID: 19109863 DOI: 10.3748/WJG.14.7302]
5 **Feuerstadt P**, Brandt LJ. Update on Colon Ischemia: Recent Insights and Advances. *Curr Gastroenterol Rep* 2015; **17**: 45 [PMID: 26446556 DOI: 10.1007/s11894-015-0469-6]
6 **Mandel LJ**, Balaban RS. Stoichiometry and coupling of active transport to oxidative metabolism in epithelial tissues. *Am J Physiol* 1981; **240**: F357-F371 [PMID: 7015879]
7 **Del Castillo JR**, Ricabarra B, Sulbarán-Carrasco MC. Intermediary metabolism and its relationship with ion transport in isolated guinea pig colonic epithelial cells. *Am J Physiol* 1991; **260**: C626-C634 [PMID: 2003584]
8 **Zheng L**, Kelly CJ, Colgan SP. Physiologic hypoxia and oxygen homeostasis in the healthy intestine. A Review in the Theme: Cellular Responses to Hypoxia. *Am J Physiol Cell Physiol* 2015; **309**: C350-C360 [PMID: 26179603 DOI: 10.1152/ajpcell.00191.2015]
9 **Suarez F**, Furne J, Springfield J, Levitt M. Insights into human colonic physiology obtained from the study of flatus composition. *Am J Physiol* 1997; **272**: G1028-G1033 [PMID: 9176210]
10 **Sahakian AB**, Jee SR, Pimentel M. Methane and the gastrointestinal tract. *Dig Dis Sci* 2010; **55**: 2135-2143 [PMID: 19830557 DOI: 10.1007/s10620-009-1012-0]
11 **Bornside GH**, Donovan WE, Myers MB. Intracolonic tensions of oxygen and carbon dioxide in germfree, conventional, and gnotobiotic rats. *Proc Soc Exp Biol Med* 1976; **151**: 437-441 [PMID: 766018]
12 **Albenberg L**, Esipova TV, Judge CP, Bittinger K, Chen J, Laughlin A, Grunberg S, Baldassano RN, Lewis JD, Li H, Thom SR, Bushman FD, Vinogradov SA, Wu GD. Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. *Gastroenterology* 2014; **147**: 1055-1063.e8 [PMID: 25046162 DOI: 10.1053/j.gastro.2014.07.020]
13 **Guebel DV**, Torres NV. A computer model of oxygen dynamics in human colon mucosa: implications in normal physiology and early tumor development. *J Theor Biol* 2008; **250**: 389-409 [PMID: 18022199]
14 **Saldeña TA**, Saraví FD, Hwang HJ, Cincunegui LM, Carra GE. Oxygen diffusive barriers of rat distal colon: role of subepithelial tissue, mucosa, and mucus gel layer. *Dig Dis Sci* 2000; **45**: 2108-2114 [PMID: 11215723]
15 **Saraví FD**, Saldeña TA, Cincunegui LM, Carra GE. Asymmetrical oxygen availability from serosal and luminal sides of rat distal colon epithelium. *Rev Esp Fisiol* 1997; **53**: 367-375 [PMID: 9580471]
16 **Siegelbaum SA**, Koester J. Ion channels. In: Kandel ER, Schwartz JH, Jessell TM, Siegelbaum SA, Hudspeth AJ. Principles of neural science. 5th ed. New York: McGraw-Hill Medical, 2013: 100-125
17 **Saraví FD**, Saldeña TA, Carrera CA, Ibáñez JE, Cincunegui LM, Carra GE. Oxygen consumption and chloride secretion in rat distal colon isolated mucosa. *Dig Dis Sci* 2003; **48**: 1767-1773 [PMID: 14560999]
18 **Carra GE**, Ibáñez, Saraví FD. Electrogenic transport, oxygen consumption, and sensitivity to acute hypoxia of human colonic epithelium. *Int J Colorectal Dis* 2011; **26**: 1205-1210 [PMID: 21519802 DOI: 10.1007/s00384-011-1215-7]
19 **Boron WF**, Waisbren SJ, Modlin IM, Geibel JP. Unique permeability barrier of the apical surface of parietal and chief cells in isolated perfused gastric glands. *J Exp Biol* 1994; **196**: 347-360 [PMID: 7823033]

20 **Endeward V**, Gros G. Low carbon dioxide permeability of the apical epithelial membrane of guinea-pig colon. *J Physiol* 2005; **567**: 253-265 [PMID: 15932894]
21 **Boron WF**. Sharpey-Schafer lecture: gas channels. *Exp Physiol* 2010; **95**: 1107-1130 [PMID: 20851859 DOI: 10.1113/expphysiol.2010.055244]
22 **Endeward V**, Al-Samir S, Itel F, Gros G. How does carbon dioxide permeate cell membranes? A discussion of concepts, results and methods. *Front Physiol* 2014; **4**: 382 [PMID: 24409149 DOI: 10.3389/fphys.2013.00382]

**P- Reviewer**: Huerta-Franco MR, Koch TR **S- Editor**: Song XX
**L- Editor**: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pathophysiology

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

*World J Gastrointest Pathophysiol* 2017 May 15; 8(2): 67-76

DOI: 10.4291/wjgp.v8.i2.67

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Basic Study

# Combination curcumin and vitamin E treatment attenuates diet-induced steatosis in *Hfe*$^{-/-}$ mice

Mandy Heritage, Lesley Jaskowski, Kim Bridle, Catherine Campbell, David Briskey, Laurence Britton, Linda Fletcher, Luis Vitetta, V Nathan Subramaniam, Darrell Crawford

Mandy Heritage, Lesley Jaskowski, Kim Bridle, Laurence Britton, V Nathan Subramaniam, Darrell Crawford, School of Medicine, The University of Queensland, Brisbane 4120, Australia

Mandy Heritage, Lesley Jaskowski, Kim Bridle, Laurence Britton, Darrell Crawford, Gallipoli Medical Research Institute, Greenslopes Private Hospital, Brisbane 4120, Australia

Catherine Campbell, Envoi Specialist Pathologists, Brisbane 4120, Australia

David Briskey, School of Human Movement and Nutrition Sciences, The University of Queensland, Brisbane 4120, Australia

Linda Fletcher, Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, Brisbane 4120, Australia

Luis Vitetta, The University of Sydney, Sydney Medical School, Sydney 2006, Australia

Luis Vitetta, Medlab Clinical Ltd, Sydney 2006, Australia

V Nathan Subramaniam, The QIMR Berghofer Medical Research Institute, Brisbane 4120, Australia

V Nathan Subramaniam, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane 4120, Australia

**Author contributions:** Heritage M, Jaskowski L and Briskey D performed all experiments for the study; Campbell C undertook histological scoring; Heritage M, Bridle K, Britton L, Fletcher L, Vitetta L, Subramaniam VN and Crawford D were responsible for study design, data analysis and manuscript editing; Heritage M and Bridle K wrote the manuscript; Subramaniam VN and Crawford D were the joint senior authors.

**Institutional review board statement:** No human subjects were used in this study.

**Institutional animal care and use committee statement:** All procedures involving animals were reviewed and approved by the Animal Ethics Committees of QIMR Berghofer Medical Research Institute (P829) and the University of Queensland (313/11).

**Conflict-of-interest statement:** The authors report no conflicts of interest.

**Data sharing statement:** No additional data are available.



**Manuscript source:** Invited manuscript

**Correspondence to: Dr. Kim Bridle,** School of Medicine, The University of Queensland, Lower Lobby Level, Administration Building, Greenslopes Private Hospital, Newdegate Street, Brisbane 4120, Australia. k.bridle@uq.edu.au
**Telephone:** +61-7-33460698
**Fax:** +61-7-38476236

**Received:** August 28, 2016
**Peer-review started:** September 1, 2016
**First decision:** October 31, 2016
**Revised:** December 1, 2016
**Accepted:** February 28, 2017
**Article in press:** March 2, 2017
**Published online:** May 15, 2017

## Abstract

***AIM***

To investigate the synergistic hepato-protective properties of curcumin and vitamin E in an *Hfe*$^{-/-}$ high calorie diet model of steatohepatitis.

***METHODS***

*Hfe*$^{-/-}$ C57BL/6J mice were fed either a high calorie diet or a high calorie diet with 1 mg/g curcumin; 1.5 mg/g vitamin E; or combination of 1 mg/g curcumin + 1.5 mg/g vitamin E for 20 wk. Serum and liver tissue were collected at the completion of the experiment. Liver histology was graded by a pathologist for steatosis, inflammation and fibrosis. RNA and protein was extracted from liver tissue to examine gene and protein expression associated with fatty acid oxidation, mitochondrial biogenesis and oxidative stress pathways.

***RESULTS***

*Hfe*$^{-/-}$ mice fed the high calorie diet developed steatohepatitis and pericentral fibrosis. Combination treatment with curcumin and vitamin E resulted in a greater reduction of percent steatosis than either vitamin E or curcumin therapy alone. Serum alanine aminotransferase and non-alcoholic fatty liver disease (NAFLD) activity score were decreased following combination therapy with curcumin and vitamin E compared with high calorie diet alone. No changes were observed in inflammatory or fibrosis markers following treatment. Epididymal fat pad weights were significantly reduced following combination therapy, however total body weight and liver weight were unchanged. Combination therapy increased the mRNA expression of *AdipoR2*, *Ppar-α*, *Cpt1a*, *Nrf-1* and *Tfb2m* suggesting enhanced fatty acid oxidation and mitochondrial biogenesis. In addition, combination treatment resulted in increased catalase activity in *Hfe*$^{-/-}$ mice.

***CONCLUSION***

Combination curcumin and vitamin E treatment decreases liver injury in this steatohepatitis model, indicating that combination therapy may be of value in NAFLD.

**Key words:** Non-alcoholic fatty liver disease; Hemochromatosis; Iron overload; Steatosis; High calorie diet



**Core tip:** The high prevalence of obesity and the metabolic syndrome suggests that many patients with liver disease of varying etiologies will have co-existent non-alcoholic fatty liver disease. Our model of co-toxic liver disease incorporates increased hepatic iron in combination with steatosis and was associated with necroinflammation and early hepatic fibrosis. Because of the beneficial effect of combination therapy, we believe vitamin E and curcumin should be investigated in other animal models of non-alcoholic steatohepatitis. Should beneficial effects be demonstrated, combination treatment with the development of appropriate dosing strategies could be rapidly moved to human studies allowing for an effective treatment strategy.

Heritage M, Jaskowski L, Bridle K, Campbell C, Briskey D, Britton L, Fletcher L, Vitetta L, Subramaniam VN, Crawford D. Combination curcumin and vitamin E treatment attenuates diet-induced steatosis in *Hfe*$^{-/-}$ mice. *World J Gastrointest Pathophysiol* 2017; 8(2): 67-76 Available from: URL: http://www.wjgnet.com/2150-5330/full/v8/i2/67.htm DOI: http://dx.doi.org/10.4291/wjgp.v8.i2.67

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in Western countries[1] and encompasses a range of hepatic pathologies from simple steatosis to the more aggressive non-alcoholic steatohepatitis (NASH)[2]. Homozygosity for the C282Y mutation in the *HFE* gene is the most common cause of hereditary hemochromatosis (HH)[3]. Steatosis is common in patients with HH[4] and is associated with increased hepatic fibrosis; conversely, heterozygosity for the C282Y mutation in *HFE* is common in patients with NAFLD[5]. Even mild increases in hepatic iron concentration (HIC) may play an important role in the transition from simple steatosis to NASH as *Hfe*$^{-/-}$ mice fed a high calorie diet develop NASH, impaired anti-oxidant activity and accelerated liver injury[6]. This relationship between HFE, altered iron metabolism and the development of NAFLD could be the target for therapeutic strategies that may attenuate disease progression in NASH. Curcumin, the yellow pigment of the plant *Curcuma longa* (turmeric), has potent antioxidant and chemo preventative effects[7]. Curcumin treatment produces beneficial effects in many animal models of liver diseases[8,9]. Curcumin ameliorates the early stages of experimental steatohepatitis and limits the development and progression of fibrosis in mice fed a methionine choline deficient diet[10,11]. In addition, a recent randomised controlled trial of curcumin in fatty liver patients demonstrated a reduction in NASH features[12]. The anti-oxidant, vitamin E has been shown to be beneficial in animal models of liver disease and in subjects with NASH[13-15].

Curcumin and vitamin E have been used with therapeutic benefit in experimental models of NASH[10,11,14]. To date, these agents have mostly been used at the initiation of injury which does not validate their utility in NASH in the usual clinical situation of already established injury and ongoing exposure to excess calorie intake. We hypothesized that these two agents may have synergistic activity in attenuating disease progression in NASH. Thus, in the present study, we investigated combination use of curcumin and vitamin E in the treatment of *Hfe*$^{-/-}$ mice with established liver injury induced by a high-fat, high-carbohydrate diet, and with ongoing exposure to the diet. Our results show that combination treatment of curcumin and vitamin E decreased liver injury and hepatic steatosis suggesting that this treatment may be of therapeutic value in NAFLD.

## MATERIALS AND METHODS

### *Animals*

All animals received humane care under the guidelines and approval of the QIMR Berghofer Medical Research

Institute and The University of Queensland Animal Ethics Committees. Eight-week-old male *Hfe*$^{-/-}$ mice (on a C57BL/6J background)[16], were fed a high calorie diet (HCD) (SF03-020, Speciality Feeds, Glen Forrest, WA, Australia) ($n$ = 34) for a period of 10 wk. The mice were then randomly assigned ($n$ = 7-9 per group) to receive either HCD alone, or HCD and 1 mg/g curcumin (Curcumin C$^3$ complex, Sabinsa Corporation, Sydney, NSW, Australia), HCD and 1.5 mg/g vitamin E or HCD and a combination of 1 mg/g curcumin and 1.5 mg/g vitamin E for a further 10 wk (Specialty Feeds). The fat composition of the HCD by weight was 150 mg/g saturated fat, 20 mg/g polyunsaturated and 60 mg/g monounsaturated fat, derived from a predominant mixture of cocoa butter (50 mg/g), partially hydrogenated vegetable oil (131 mg/g) and canola oil (50 mg/g) with added cholesterol (1.9 mg/g). Animals were allowed *ad libitum* access to diets and drinking water. After 20 wk of dietary treatment, animals were sacrificed under anaesthesia following cardiac puncture for blood collection. The livers were removed and portions were snap-frozen in liquid nitrogen and stored at -80 ℃ prior to analysis. Separate portions were collected for histology or dried for the determination of hepatic iron concentration (HIC). Epididymal fat pads were weighed and stored at -80 ℃.

### Histopathological analysis

Liver samples were fixed in 40 g/L neutral-buffered formalin, embedded in paraffin, sectioned and stained with haematoxylin-eosin (H and E). Histological parameters were staged and graded according to accepted criteria by a specialist liver pathologist in a blinded fashion[17]. Steatosis was graded according to the percentage of steatotic hepatocytes (grade 0, < 5% affected; grade 1, 5%-33% affected; grade 2, 34%-66% affected; and grade 3, > 66% affected). Lobular inflammatory activity was scored based on the number of inflammatory foci per 200x field (0, none; 1, < 2 seen; 2, 2-4 seen; and 3, > 4 seen) and ballooning was scored based on the degree of hepatocyte ballooning (0, none; 1, few; and 2, many). Activity was also scored using NAFLD activity score (NAS) established by the NASH Clinical Research Network (CRN), which is an unweighted sum of scores for steatosis (0-3), lobular inflammation (0-3) and ballooning (0-2)[17].

Fibrosis stage was assessed following Picro Sirius Red staining for collagen according to the criteria established by Brunt *et al*[18].

### Serum biochemistry and hepatic iron concentration

Serum concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured on a Cobas Integra 400 Chemistry Automated Analyser as per manufacturer's instructions (Roche Diagnostics, Castle Hill, NSW, Australia). HIC was measured as previously described[6,19].

### Hepatic antioxidant enzyme assay

Total cellular glutathione peroxidase (GPx), reduced (GSH) and oxidized glutathione (GSSG), catalase and mitochondrial manganese superoxide dismutase (MnSOD) activities were measured on homogenized liver tissue using commercial assay kits as per manufacturer's instructions (Cayman Chemical, Ann Arbor, MI, United States).

### RNA extraction and quantitative real-time polymerase chain reaction

Total RNA was extracted using TRIzol reagent (Invitrogen, Mount Waverley, Victoria, Australia), subjected to deoxyribonuclease Ⅰ digestion and transcribed into cDNA using Superscript Ⅲ according to the manufacturer's instructions (Invitrogen). Quantitative gene expression was performed by real-time polymerase chain reaction (RT-PCR) (ViiA™ 7, Applied Biosystems, Life Technologies Corporation, Carlsbad, CA, United States) using Quantifast SYBR green as per manufacturer's conditions (Qiagen, Chadstone Centre, VIC, Australia). The expression of individual genes were normalised to the geometric mean of three house-keeper genes: Basic transcription factor 3 (*Btf-3*), Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh)* and β2-microglobulin (*β2-m*). Mouse primer sequences for genes investigated are shown in Supplementary Table 1.

### Western blot and densitometry analysis

Protein extracts (30 μg) from liver were electrophoresed in sodium dodecyl sulphate-10% polyacrylamide gel for 30 min at 100 V and blotted onto polyvinylidene fluoride membranes (Bio-Rad, Hercules, CA, United States) as previously described[6]. Membranes were immunostained with primary antibodies for ACOX (Abcam, Cambridge, MA, United States) and mitoNEET (Proteintech, Chicago, IL, United States). Signals were detected using standard chemiluminescence (Supersignal West Femto, ThermoFisher Scientific, Scoresby, Victoria, Australia) on an Image Station 4000MM Pro and quantified using Carestream Molecular Imaging software (Carestream Australia, East Melbourne, Victoria, Australia).

### Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 22 (IBM, Armonk, NY, United States). Non-parametric tests were used to evaluate differences between groups, and data are depicted using box and whisker plots showing median, minimum and maximum values. A Kruskal-Wallis test with Dunn's correction for multiple comparisons was performed to compare intervention groups. A cut-off $P$ value of 0.05 was used to determine significance. Statistical review of the manuscript was performed by a biomedical statistician.

## RESULTS

### Serum transaminase concentrations are decreased by curcumin and vitamin E combination therapy

Total body weight and liver weight were not altered by dietary treatment in *Hfe*$^{-/-}$ mice (Figure 1A). Epididymal

**Table 1 Percent steatosis, lobular and non-alcoholic fatty liver disease activity score were reduced due to combination therapy**

| | HCD ($n$ = 9) | HCD + Cu ($n$ = 9) | HCD + VE ($n$ = 7) | HCD + Cu + VE ($n$ = 9) |
|---|---|---|---|---|
| Histology diagnosis | NASH (9) | Steatosis (2)/NASH (7) | Steatosis (2)/NASH (5) | Steatosis (4)/NASH (5) |
| Steatosis grade | 3 (3-3) | 3 (2-3) | 3 (2-3) | 3 (2-3) |
| % steatosis | 95 (80-100) | 80 (40-100) | 95 (70-100) | 75 (40-95)[a] |
| Lobular score | 2 (1-2) | 2 (1-3) | 1 (0-2) | 1 (0-3) |
| Ballooning | 2 (1-2) | 2 (1-2) | 1 (1-2) | 1 (0-2) |
| NAS score | 6 (5-7) | 6 (4-8) | 6 (4-6) | 5 (3-8) |
| Fibrosis stage | 1a (0-1a) | 0 (0-1a) | 0 (0-1a) | 0 (0-1a) |

Values are expressed as the median (range). [a]$P < 0.05$ *vs* HCD alone, Kruskal-Wallis ANOVA. HCD: High calorie diet; Cu: Curcumin; VE: Vitamin E; NAS: Non-alcoholic fatty liver disease activity score.

fat pad weight was significantly reduced in the combination treatment group compared to curcumin alone ($P < 0.02$, Figure 1A).

Serum ALT concentrations were significantly reduced by combination treatment compared to HCD alone ($P = 0.02$, Figure 1B). AST concentrations were not significantly different across groups ($P = 0.12$). There was no significant effect of dietary treatment on HIC ($P < 0.30$, Figure 1B).

### *Attenuation of severe steatosis and lobular inflammation by curcumin and vitamin E combination therapy*

*Hfe*$^{-/-}$ HCD-fed mice developed moderate to severe steatosis with hepatocyte ballooning and inflammatory cell infiltration present in 100% of mice meeting the criteria for steatohepatitis (Table 1 and Figure 2A). Combination treatment with curcumin and vitamin E reduced the presence of NASH to only 55% of mice. Combination treatment also significantly reduced percent steatosis compared with HCD alone ($P = 0.03$). Lobular inflammatory score and NAS score were also reduced in combination treated *Hfe*$^{-/-}$ mice (Table 1 and Figure 2A). Treatment with either curcumin or vitamin E alone had minimal effects on hepatic histology (Figure 2A).

Centrilobular (stage 1) fibrosis (as detected by Sirius red staining) was observed in 7/9 *Hfe*$^{-/-}$ mice fed the HCD and only in 3/9 combination treated mice, as evidenced by reduced staining (data not shown). However, hepatic $\alpha_1$(I)-procollagen (*Col1a1*) mRNA, transforming growth factor $\beta_1$ (*Tgf$\beta$1*) and tissue inhibitor of metalloproteinase 1 (*Timp1*) gene expression were not significantly altered by dietary treatment ($P = 0.05$, $P = 0.40$ and $P = 0.26$ respectively, Figure 2B). Gene expression of monocyte chemoattractant protein-1 (*Mcp-1*) and two acute phase reactants, orosomucoid 1 (*Orm-1*) and serum amyloid A1 (*Saa1*) were not significantly altered by combination treatment when compared to HCD or monotherapy ($P = 0.08$, $P = 0.07$ and $P = 0.17$ respectively, Figure 2C).

### *Enhanced gene expression of fatty acid oxidation pathways with curcumin and vitamin E combination therapy*

Hepatic adiponectin receptor 2 (*AdipoR2*) mRNA expression was significantly increased in vitamin E and combination treatment groups compared to HCD alone ($P = 0.02$ and $P < 0.01$, respectively).

Downstream of *AdipoR2*, peroxisome proliferator-activated receptor α (*Pparα*) mRNA expression was significantly increased in combination treated *Hfe*$^{-/-}$ mice compared to HCD alone and curcumin alone ($P < 0.012$ and $P = 0.01$, respectively, Figure 3A). Carnitine palmitoyl transferase 1A (*Cpt1a*) expression was also increased due to dietary treatments where vitamin E and combination treatment significantly increased *Cpt1a* expression in *Hfe*$^{-/-}$ mice compared to HCD alone ($P = 0.05$ and $P < 0.01$, respectively, Figure 3A). In addition, combination therapy increased *Cpt1a* expression above curcumin treatment alone ($P < 0.01$, Figure 3A). The expression of acyl-coenzymeA oxidase 1 (ACOX1) protein, the first enzyme in the β-oxidation pathway, was significantly different following dietary intervention. Combination treatment up-regulated ACOX1 expression compared with HCD alone ($P < 0.01$, Figure 3B). These results suggest an increase in lipid metabolism by β-oxidation which has been altered by long-term HCD feeding.

### *Effects on hepatic antioxidant enzyme activities and mitochondrial function*

Neither glutathione peroxidase activity (GPx) nor hepatic mitochondrial manganese superoxide dismutase (MnSOD) activity were significantly altered by dietary treatments ($P = 0.4$ and $P = 0.80$, respectively, Figure 4A). However both curcumin and combination treatment increased catalase when compared to HCD alone ($P = 0.01$ and $P < 0.01$, respectively, Figure 4A).

We have previously shown mitochondrial dysfunction in *Hfe*$^{-/-}$ mice fed a HCD[6], therefore we then investigated if combination treatment could correct this defect. Indeed, nuclear transcription factor 1 (*Nrf-1*) mRNA expression was significantly altered by dietary treatments in *Hfe*$^{-/-}$ mice (Figure 4B), where vitamin E and combination treatment significantly increased expression compared to HCD alone ($P = 0.01$ and $P < 0.01$, respectively). Likewise, transcription factor B2 mitochondrial (*TfB2M*) mRNA was significantly increased in *Hfe*$^{-/-}$ mice treated with vitamin E and combination treatment compared to HCD ($P < 0.01$ and $P < 0.01$, respectively, Figure 4B). These results suggest an up-regulation in mitochondrial biogenesis due to vitamin

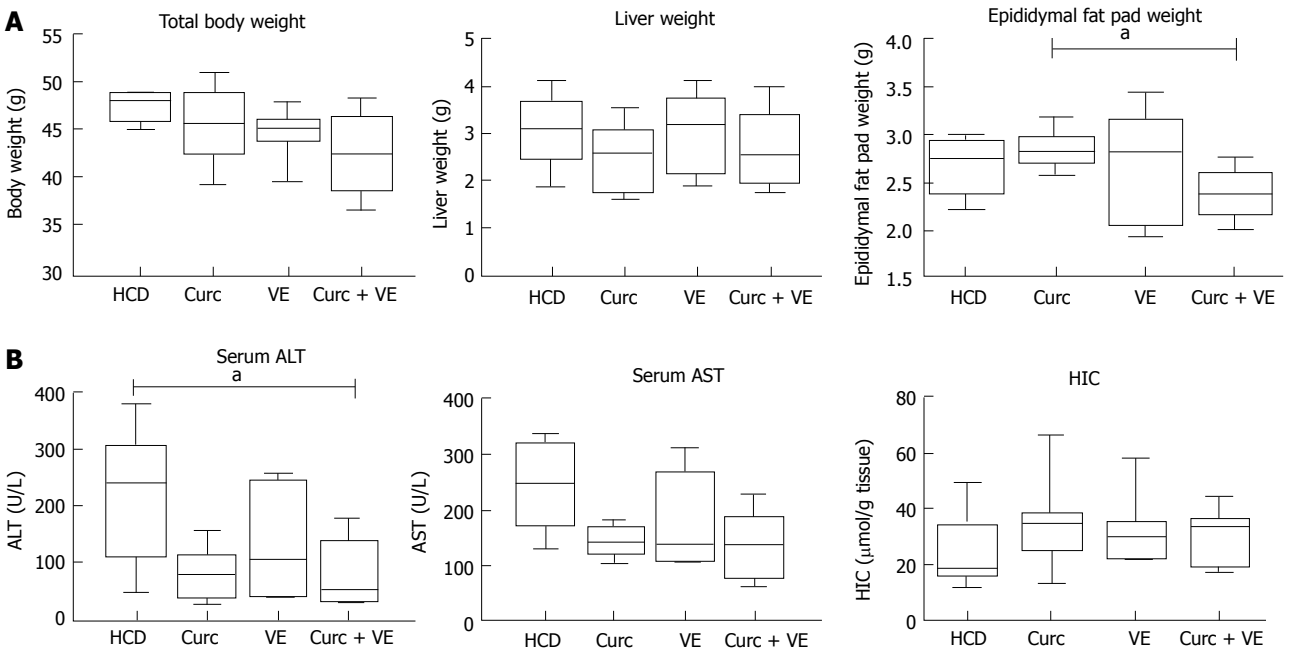


**Figure 1 Epididymal fat pad weights and serum transaminase concentrations are decreased by curcumin and vitamin E combination therapy.** *Hfe*$^{-/-}$ mice were fed HCD for 10 wk then HCD + Cu, HCD + VE or HCD + Cu + VE for a further 10 wk ($n$ = 7-9). A: Body, liver and epididymal fat pad weights were determined. B: Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations were determined on final cardiac bleeds. Following sacrifice, a dried liver segment was digested to determine hepatic iron concentration (HIC). Data are depicted using box and whisker plots showing median, minimum and maximum values. Statistical significance was tested using Kruskal-Wallis test with Dunn's correction for multiple comparisons, $^{a}P \leqslant 0.05$. HCD: High calorie diet; Cu: Curcumin; VE: Vitamin E.

E and combination treatment. Interestingly, mitoNEET, a protein that is a driving force behind mitochondrial biogenesis[20], was not significantly different in combination treated animals compared with HCD alone (Figure 4C).

## DISCUSSION

In this study we demonstrated that the combination of curcumin and vitamin E therapy attenuated steatosis and lobular inflammation in *Hfe*$^{-/-}$ mice even with ongoing feeding of a HCD. *Hfe*$^{-/-}$ mice fed a HCD for 20 wk developed steatosis and 100% had histological features consistent with steatohepatitis and the majority of animals showed centrilobular fibrosis. Monotherapy with either vitamin E or curcumin had no effect on percent steatosis, but combination therapy reduced percent hepatic steatosis as well as lobular inflammation, ballooning degeneration and fibrosis. This implies that vitamin E and curcumin exert a synergistic effect over that provided by each individual therapy.

Our previous studies have shown that *Hfe*$^{-/-}$ mice fed a HCD develop steatosis and the pathophysiology of this injury involves altered β-oxidation, impaired fatty acid uptake and mitochondrial dysfunction[6]. To assess the mechanisms through which curcumin and vitamin E may exert their beneficial effect we examined fatty acid uptake, β-oxidation and mitochondrial function in this study.

Curcumin has been reported to increase the metabolism of lipids by β-oxidation[21,22] and this appears to be a mechanism by which combination therapy results in reduced hepatic steatosis in this study. This was evidenced by increased *AdipoR2, Ppar-α* and *Cpt1a* mRNA expression in the combination fed *Hfe*$^{-/-}$ mice; facilitating increased mitochondrial uptake of free fatty acids. *AdipoR2* has been shown to activate *Ppar-α* and fatty acid oxidation genes[23], and these results imply that combination therapy induced *AdipoR2* expression, resulting in the up-regulation of fatty acid oxidation pathways[24]. However, it is worth noting that the change in *AdipoR2 and Cpt1* expression with vitamin E monotherapy alone was similar to that achieved by combination therapy. Increased ACOX1 expression, facilitating increased β-oxidation, was also observed in combination treated animals, providing further evidence of increased fatty acid β-oxidation.

We examined defence mechanisms against oxidative stress as another potential reason for the benefits of combination therapy since both curcumin and vitamin E are potent anti-oxidants. Curcumin and combination treatment in *Hfe*$^{-/-}$ mice raised catalase activity illustrating that an up-regulation in β-oxidation was counteracted by an up-regulation of an enzyme that removes excess reactive oxygen species. These observations are consistent with other models of hepatic injury where the anti-oxidant effects of curcumin and vitamin E are mediated by altering catalase and MnSOD activity[25,26].

Vitamin E and combination treatment increased gene expression of *Nrf-1* and *Tfb2m* which are consistent with up-regulated mitochondrial biogenesis. It has been

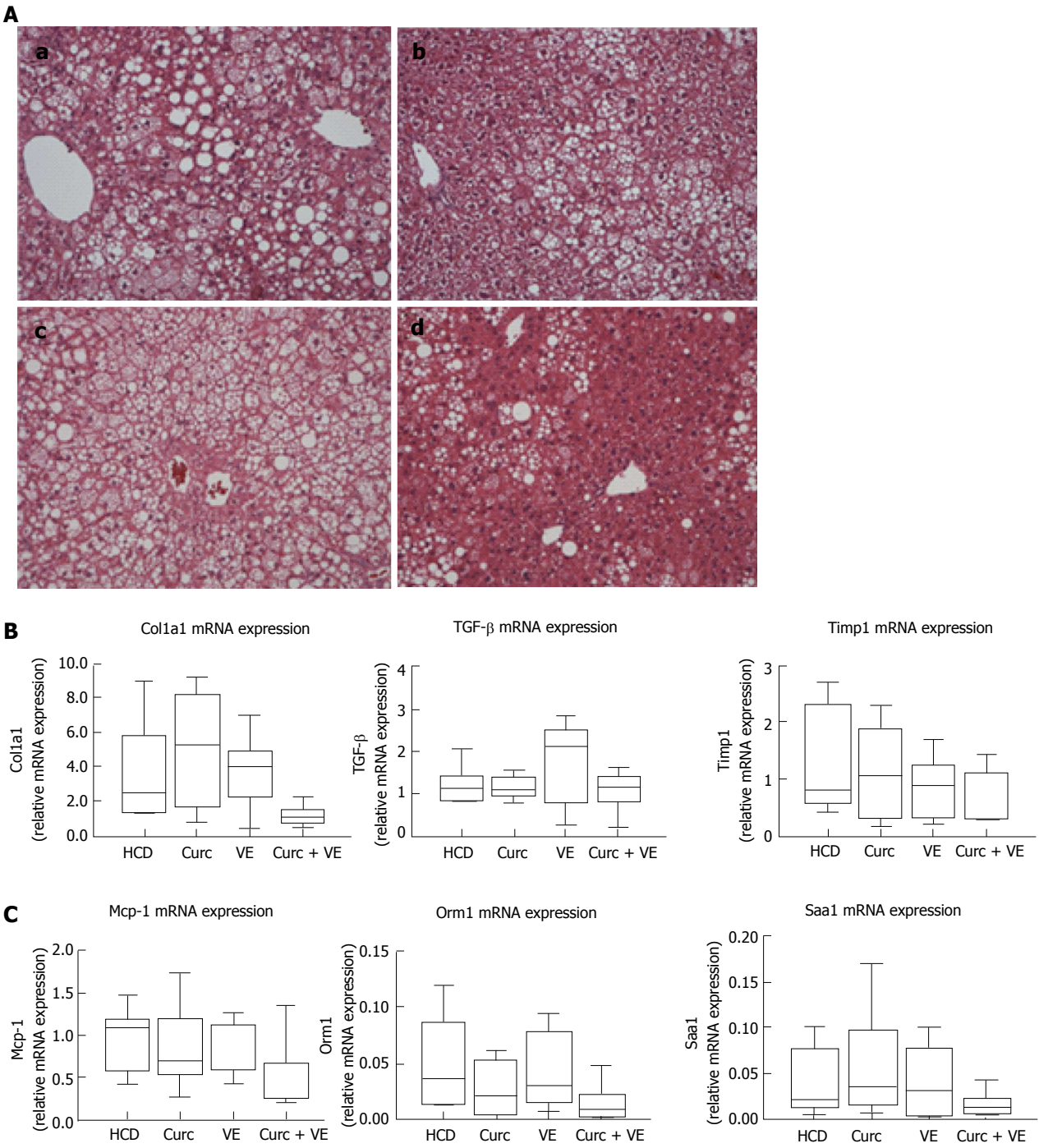


**Figure 2 Steatosis is attenuated by curcumin and vitamin E combination therapy while the expression of fibrotic and inflammatory genes are not significantly altered.** A: Representative liver paraffin-embedded sections stained with hematoxylin and eosin (H and E) (original magnification × 20). a: *Hfe*$^{-/-}$ mice (n = 7-9 per group) fed HCD (20 wk); b: HCD (10 wk) then HCD + 1% curcumin (10 wk); c: HCD (10 wk) then HCD + 1.5% vitamin E (10 wk); d: HCD (10 wk) then HCD + 1% curcumin + 1.5% vitamin E (10 wk). qRT-PCR was used to determine expression levels of hepatic (B) fibrogenic genes: α1(I)-procollagen (Col1a1); Transforming growth factor β (TGF-β); Tissue inhibitor of metalloproteinase 1 (Timp1); C: Hepatic inflammatory genes: monocyte chemoattractant protein-1 (Mcp-1); orosmucoid 1 (Orm1) and serum amyloid A 1 (Saa1). Data are depicted using box and whisker plots showing median, minimum and maximum values. Statistical significance was tested using Kruskal-Wallis test with Dunn's correction for multiple comparisons. HCD: High calorie diet; Cu: Curcumin; VE: Vitamin E.

suggested that curcumin may be similar to resveratrol in this respect through activating PPARγ coactivator 1-alpha (PGC-1α)[21,22], a regulator of mitochondrial biogenesis[27]. Indeed, curcumin increases PGC-1α and *Nrf-1* gene expression *in vitro*, protecting against mitochondrial impairment induced by high free fatty acids[28]. A recent study in a rat model of NASH demonstrated attenuation of liver injury by curcumin which the authors suggested was Nrf-1 mediated[29]. Combination treatment increased *Nrf-1* and TfB2m expression in *Hfe*$^{-/-}$ mice but failed to increase the expression of mitoNEET. These results are similar to MnSOD activity where combination treatment failed to restore its activity above a HCD-induced suppression. Down-regulation of mitoNEET causes

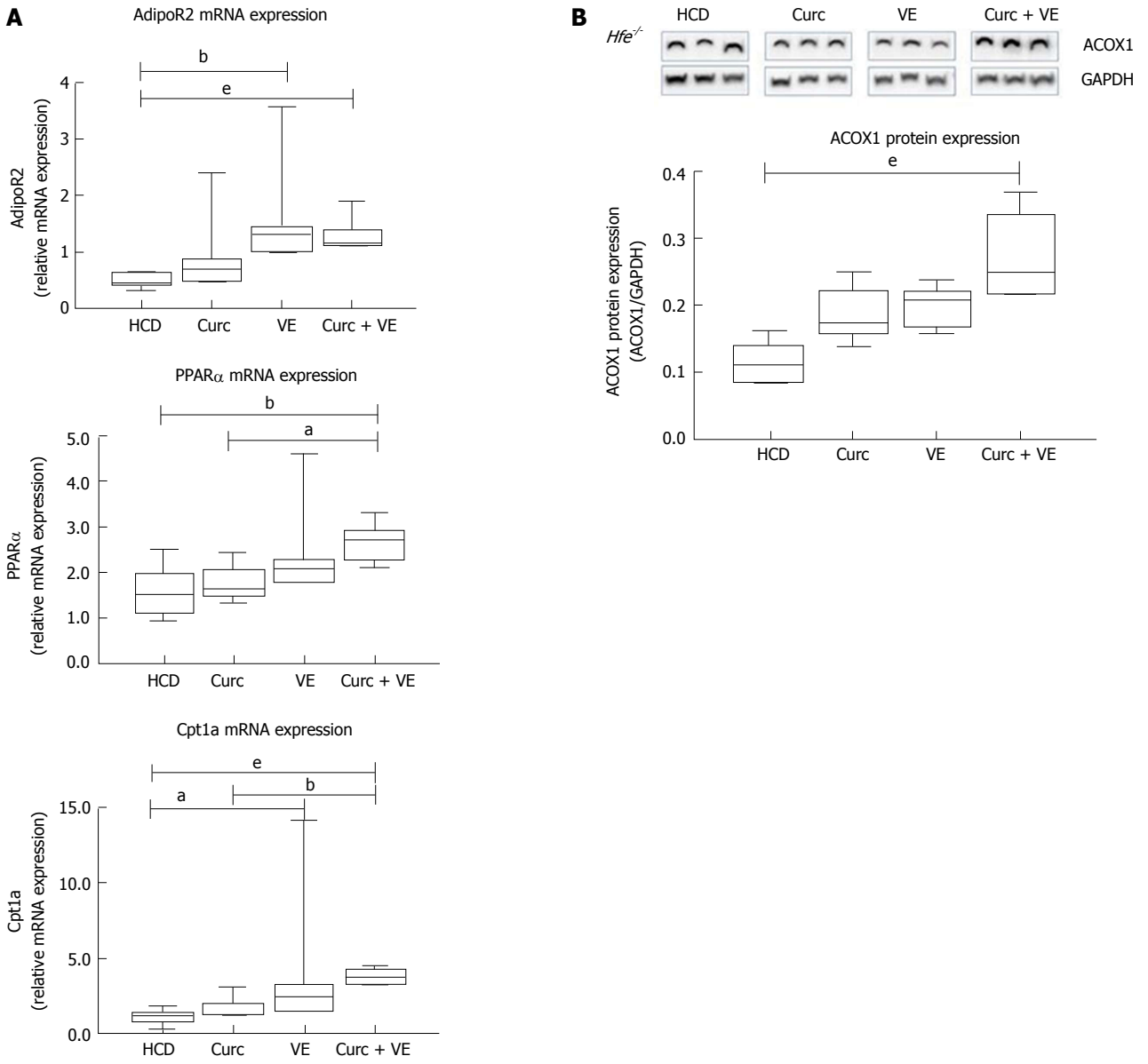


**Figure 3 Altered lipid signalling following high calorie diet feeding is abrogated by curcumin and vitamin E combination therapy.** qRT-PCR was used to determine expression levels of hepatic fatty acid oxidation genes in *Hfe*$^{-/-}$ mice fed HCD, HCD + Cu, HCD + VE or HCD + Cu + VE. A: Adiponectin receptor 2 (AdipoR2) mRNA; peroxisome proliferator-activated receptor α (PPARα) and carnitine palmitoyl transferase 1A (Cpt1a) mRNA; B: Western blotting and densitometry analysis was performed to determine levels of acyl-coenzymeA oxidase 1 (ACOX) and glyceraldehyde 3-phosphate dehydrogenase (Gapdh). Data are depicted using box and whisker plots showing median, minimum and maximum values. Statistical significance was tested using Kruskal-Wallis test with Dunn's correction for multiple comparisons, $^{a}P \leqslant 0.05$, $^{b}P \leqslant 0.01$, $^{e}P \leqslant 0.001$. HCD: High calorie diet; Cu: Curcumin; VE: Vitamin E.

an increase in iron content into the mitochondria and decreases the mitochondria's capacity to carry out electron transport and oxidative phosphorylation[20], potentially increasing oxidative stress and further damaging mitochondria. Indeed, mitochondrial dysfunction is associated with insulin resistance and the development of type 2 diabetes where diminished oxidative capacity is thought to be involved in disease pathogenesis[30]. Taken together, these findings of our study suggest that combination treatment manages to enhance the capacity for fatty acid disposal by β-oxidation without increasing oxidative stress.

This study is limited by the use of an animal model which cannot fully replicate the pathophysiology of human NASH. The dietary model incorporated high levels of curcumin which may not be easily replicated in humans. Further studies examining dosing of curcumin and vitamin E in humans are still required. While these studies have suggested potential mechanistic pathways involved in efficacy of combination treatment, we have not fully elucidated mechanisms responsible for the synergistic effects of combination therapy over monotherapy.

The high prevalence of obesity and the metabolic syndrome suggests that many patients with liver disease of varying etiologies will have co-existent non-alcoholic fatty liver disease. Indeed contemporary clinical practice in hepatology is often characterised by

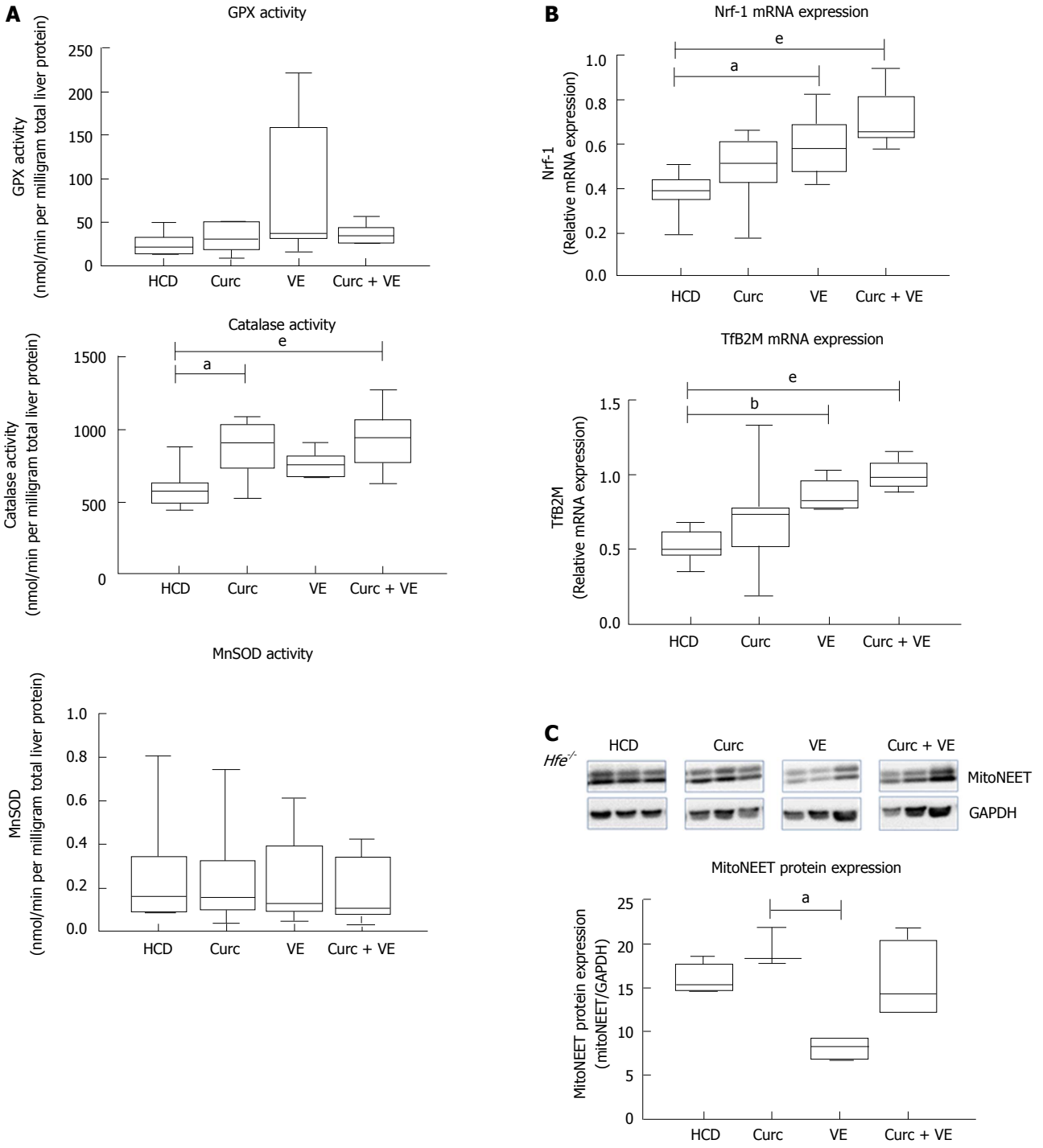


**Figure 4 Curcumin and vitamin E treatments increase hepatic antioxidant enzyme activities and increase mitochondrial biogenesis.** Hepatic tissue from Hfe-/- mice fed HCD for 10 wk then HCD + Cu, HCD + VE or HCD + Cu + VE for a further 10 wk ($n$ = 7-9) was analysed for (A) glutathione peroxidase (GPx) activity, catalase activity and mitochondrial superoxide dismutase (MnSOD) activity. qRT-PCR was used to determine expression levels of hepatic (B) nuclear transcription factor 1 (Nrf-1) and transcription factor B2 mitochondrial (TfB2M). C: Western blotting and densitometry analysis was performed to determine levels of hepatic mitoNEET and glyceraldehyde 3-phosphate dehydrogenase (Gapdh). Data are depicted using box and whisker plots showing median, minimum and maximum values. Statistical significance was tested using Kruskal-Wallis test with Dunn's correction for multiple comparisons, $^{a}P \leqslant 0.05$, $^{b}P \leqslant 0.01$, $^{e}P \leqslant 0.001$. HCD: High calorie diet; Cu: Curcumin; VE: Vitamin E.

the need to address multiple co-toxins in one patient. The results of the present study illustrate of the concept of co-toxic liver disease since our model incorporates increased HIC in combination with steatosis and was associated with necroinflammation and early hepatic fibrosis. Curcumin and vitamin E therapy resulted in attenuation of steatosis through increased fatty acid β-oxidation, increased catalase activity and upregulated mitochondrial biogenesis. Because of the beneficial effect of combination therapy, we believe vitamin E and curcumin should be investigated in other animal models of NASH, and could be moved rapidly into human

studies if a beneficial effect is demonstrated, and if appropriate dosing strategies can be developed.

## ACKNOWLEDGMENTS

Associate Professor Graeme Macdonald from the Gastroenterology and Hepatology unit of the Princess Alexandra Hospital for his knowledge and continual input into the project. Dr. Sarah McLeay for her review of the statistical methods in the manuscript.

## COMMENTS

### Background

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in Western countries and encompasses a range of hepatic pathologies from simple steatosis to the more aggressive non-alcoholic steatohepatitis (NASH). Homozygosity for the C282Y mutation in the *HFE* gene is the most common cause of hereditary hemochromatosis (HH). Steatosis is common in patients with HH and is associated with increased hepatic fibrosis; conversely, heterozygosity for the C282Y mutation in HFE is common in patients with NAFLD. Even mild increases in hepatic iron concentration (HIC) may play an important role in the transition from simple steatosis to NASH. Curcumin and vitamin E have been used with therapeutic benefit in experimental models of NASH, therefore this study investigated the role of curcumin and vitamin E in ameliorating injury in a model of iron overload and fatty liver.

### Research frontiers

Therapies for NASH and fatty liver disease are lacking and due to the growing prevalence of fatty liver disease are increasing important for treatment of this growing number of patients.

### Innovations and breakthrough

This study utilised combination treatment with curcumin and vitamin E which differed from previous studies which only examined monotherapy. In addition, this study examined the efficacy of treatment in established fatty liver disease, not solely at the initiation of injury.

### Applications

As both curcumin and vitamin E have been used clinically the authors suggest that combination treatment could be easily moved into human clinical trials.

### Terminology

Non-alcoholic fatty liver disease: Encompasses a range of hepatic pathologies from simple steatosis to the more aggressive non-alcoholic steatohepatitis; Haemochromatosis: An iron-overload disease which results in hepatic iron accumulation.

### Peer-review

Manuscript's content is interesting, overall well written and timely.

## REFERENCES

1 **Ong JP**, Younossi ZM. Epidemiology and natural history of NAFLD and NASH. *Clin Liver Dis* 2007; **11**: 1-16, vii [PMID: 17544968 DOI: 10.1016/j.cld.2007.02.009]
2 **Smith BW**, Adams LA. Non-alcoholic fatty liver disease. *Crit Rev Clin Lab Sci* 2011; **48**: 97-113 [PMID: 21875310 DOI: 10.3109/10408363.2011.596521]
3 **Valenti L**, Fracanzani AL, Bugianesi E, Dongiovanni P, Galmozzi E, Vanni E, Canavesi E, Lattuada E, Roviaro G, Marchesini G, Fargion S. HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology* 2010; **138**: 905-912 [PMID: 19931264 DOI: 10.1053/j.gastro.2009.11.013]
4 **Powell EE**, Ali A, Clouston AD, Dixon JL, Lincoln DJ, Purdie DM, Fletcher LM, Powell LW, Jonsson JR. Steatosis is a cofactor in liver injury in hemochromatosis. *Gastroenterology* 2005; **129**: 1937-1943 [PMID: 16344062 DOI: 10.1053/j.gastro.2005.09.015]
5 **Nelson JE**, Bhattacharya R, Lindor KD, Chalasani N, Raaka S, Heathcote EJ, Miskovsky E, Shaffer E, Rulyak SJ, Kowdley KV. HFE C282Y mutations are associated with advanced hepatic fibrosis in Caucasians with nonalcoholic steatohepatitis. *Hepatology* 2007; **46**: 723-729 [PMID: 17680648 DOI: 10.1002/hep.21742]
6 **Tan TC**, Crawford DH, Jaskowski LA, Murphy TM, Heritage ML, Subramaniam VN, Clouston AD, Anderson GJ, Fletcher LM. Altered lipid metabolism in Hfe-knockout mice promotes severe NAFLD and early fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2011; **301**: G865-G876 [PMID: 21817060 DOI: 10.1152/ajpgi.00150.2011]
7 **Sharma RA**, Steward WP, Gescher AJ. Pharmacokinetics and pharmacodynamics of curcumin. *Adv Exp Med Biol* 2007; **595**: 453-470 [PMID: 17569224 DOI: 10.1007/978-0-387-46401-5_20]
8 **Baghdasaryan A**, Claudel T, Kosters A, Gumhold J, Silbert D, Thüringer A, Leski K, Fickert P, Karpen SJ, Trauner M. Curcumin improves sclerosing cholangitis in Mdr2-/- mice by inhibition of cholangiocyte inflammatory response and portal myofibroblast proliferation. *Gut* 2010; **59**: 521-530 [PMID: 20332524 DOI: 10.1136/gut.2009.186528]
9 **Rong S**, Zhao Y, Bao W, Xiao X, Wang D, Nussler AK, Yan H, Yao P, Liu L. Curcumin prevents chronic alcohol-induced liver disease involving decreasing ROS generation and enhancing antioxidative capacity. *Phytomedicine* 2012; **19**: 545-550 [PMID: 22445643 DOI: 10.1016/j.phymed.2011.12.006]
10 **Leclercq IA**, Farrell GC, Sempoux C, dela Peña A, Horsmans Y. Curcumin inhibits NF-kappaB activation and reduces the severity of experimental steatohepatitis in mice. *J Hepatol* 2004; **41**: 926-934 [PMID: 15582125 DOI: 10.1016/j.jhep.2004.08.010]
11 **Vizzutti F**, Provenzano A, Galastri S, Milani S, Delogu W, Novo E, Caligiuri A, Zamara E, Arena U, Laffi G, Parola M, Pinzani M, Marra F. Curcumin limits the fibrogenic evolution of experimental steatohepatitis. *Lab Invest* 2010; **90**: 104-115 [PMID: 19901911 DOI: 10.1038/labinvest.2009.112]
12 **Rahmani S**, Asgary S, Askari G, Keshvari M, Hatamipour M, Feizi A, Sahebkar A. Treatment of Non-alcoholic Fatty Liver Disease with Curcumin: A Randomized Placebo-controlled Trial. *Phytother Res* 2016; **30**: 1540-1548 [PMID: 27270872 DOI: 10.1002/ptr.5659]
13 **Harrison SA**, Torgerson S, Hayashi P, Ward J, Schenker S. Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2003; **98**: 2485-2490 [PMID: 14638353 DOI: 10.1111/j.1572-0241.2003.08699.x]
14 **Chung MY**, Yeung SF, Park HJ, Volek JS, Bruno RS. Dietary α- and γ-tocopherol supplementation attenuates lipopolysaccharide-induced oxidative stress and inflammatory-related responses in an obese mouse model of nonalcoholic steatohepatitis. *J Nutr Biochem* 2010; **21**: 1200-1206 [PMID: 20138495 DOI: 10.1016/j.jnutbio.2009.10.006]
15 **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685 [PMID: 20427778 DOI: 10.1056/NEJMoa0907929]
16 **Zhou XY**, Tomatsu S, Fleming RE, Parkkila S, Waheed A, Jiang J, Fei Y, Brunt EM, Ruddy DA, Prass CE, Schatzman RC, O'Neill R, Britton RS, Bacon BR, Sly WS. HFE gene knockout produces mouse model of hereditary hemochromatosis. *Proc Natl Acad Sci USA* 1998; **95**: 2492-2497 [PMID: 9482913]
17 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/

hep.20701]
18 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474 [PMID: 10484010 DOI: 10.1111/j.1572-0241.1999.01377.x]
19 **Stuart KA**, Fletcher LM, Clouston AD, Lynch SV, Purdie DM, Kerlin P, Crawford DH. Increased hepatic iron and cirrhosis: no evidence for an adverse effect on patient outcome following liver transplantation. *Hepatology* 2000; **32**: 1200-1207 [PMID: 11093725 DOI: 10.1053/jhep.2000.20348]
20 **Kusminski CM**, Holland WL, Sun K, Park J, Spurgin SB, Lin Y, Askew GR, Simcox JA, McClain DA, Li C, Scherer PE. MitoNEET-driven alterations in adipocyte mitochondrial activity reveal a crucial adaptive process that preserves insulin sensitivity in obesity. *Nat Med* 2012; **18**: 1539-1549 [PMID: 22961109 DOI: 10.1038/nm.2899]
21 **Chung JH**, Manganiello V, Dyck JR. Resveratrol as a calorie restriction mimetic: therapeutic implications. *Trends Cell Biol* 2012; **22**: 546-554 [PMID: 22885100 DOI: 10.1016/j.tcb.2012.07.004]
22 **Zingg JM**, Hasan ST, Meydani M. Molecular mechanisms of hypolipidemic effects of curcumin. *Biofactors* 2013; **39**: 101-121 [PMID: 23339042 DOI: 10.1002/biof.1072]
23 **Yamauchi T**, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, Okada-Iwabu M, Kawamoto S, Kubota N, Kubota T, Ito Y, Kamon J, Tsuchida A, Kumagai K, Kozono H, Hada Y, Ogata H, Tokuyama K, Tsunoda M, Ide T, Murakami K, Awazawa M, Takamoto I, Froguel P, Hara K, Tobe K, Nagai R, Ueki K, Kadowaki T. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat Med* 2007; **13**: 332-339 [PMID: 17268472 DOI: 10.1038/nm1557]
24 **Handa P**, Maliken BD, Nelson JE, Morgan-Stevenson V, Messner DJ, Dhillon BK, Klintworth HM, Beauchamp M, Yeh MM, Elfers CT, Roth CL, Kowdley KV. Reduced adiponectin signaling due to weight gain results in nonalcoholic steatohepatitis through impaired mitochondrial biogenesis. *Hepatology* 2014; **60**: 133-145 [PMID: 24464605 DOI: 10.1002/hep.26946]
25 **Messarah M**, Amamra W, Boumendjel A, Barkat L, Bouasla I, Abdennour C, Boulakoud MS, Feki AE. Ameliorating effects of curcumin and vitamin E on diazinon-induced oxidative damage in rat liver and erythrocytes. *Toxicol Ind Health* 2013; **29**: 77-88 [PMID: 22609857 DOI: 10.1177/0748233712446726]
26 **Subudhi U**, Chainy GB. Curcumin and vitamin E modulate hepatic antioxidant gene expression in PTU-induced hypothyroid rats. *Mol Biol Rep* 2012; **39**: 9849-9861 [PMID: 22733496 DOI: 10.1007/s11033-012-1851-1]
27 **Jones AW**, Yao Z, Vicencio JM, Karkucinska-Wieckowska A, Szabadkai G. PGC-1 family coactivators and cell fate: roles in cancer, neurodegeneration, cardiovascular disease and retrograde mitochondria-nucleus signalling. *Mitochondrion* 2012; **12**: 86-99 [PMID: 21983689 DOI: 10.1016/j.mito.2011.09.009]
28 **Kuo JJ**, Chang HH, Tsai TH, Lee TY. Curcumin ameliorates mitochondrial dysfunction associated with inhibition of gluconeogenesis in free fatty acid-mediated hepatic lipoapoptosis. *Int J Mol Med* 2012; **30**: 643-649 [PMID: 22692588 DOI: 10.3892/ijmm.2012.1020]
29 **Li B**, Wang L, Lu Q, Da W. Liver injury attenuation by curcumin in a rat NASH model: an Nrf2 activation-mediated effect? *Ir J Med Sci* 2016; **185**: 93-100 [PMID: 25385666 DOI: 10.1007/s11845-014-1226-9]
30 **Paddock ML**, Wiley SE, Axelrod HL, Cohen AE, Roy M, Abresch EC, Capraro D, Murphy AN, Nechushtai R, Dixon JE, Jennings PA. MitoNEET is a uniquely folded 2Fe 2S outer mitochondrial membrane protein stabilized by pioglitazone. *Proc Natl Acad Sci USA* 2007; **104**: 14342-14347 [PMID: 17766440 DOI: 10.1073/pnas.0707189104]

**P- Reviewer**: Trovato GM **S- Editor**: Gong ZM **L- Editor**: A
**E- Editor**: Wu HL

World Journal of Gastrointestinal Pathophysiology

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

World J Gastrointest Pathophysiol 2017 May 15; 8(2): 77-86

DOI: 10.4291/wjgp.v8.i2.77

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Observational Study

# Endoscopic therapy for biliary strictures complicating living donor liver transplantation: Factors predicting better outcome

Harshavardhan B Rao, Hasim Ahamed, Suprabha Panicker, Surendran Sudhindran, Rama P Venu

Harshavardhan B Rao, Hasim Ahamed, Suprabha Panicker, Rama P Venu, Department of Gastroenterology, Amrita Institute of Medical Sciences, Kochi, Kerala 682041, India

Surendran Sudhindran, Department of Gastrointestinal Surgery, Amrita Institute of Medical Sciences, Kochi, Kerala 682041, India

Author contributions: Ahamed H and Panicker S helped in subject recruitment and data entry of the study; Rao HB and Venu RP were involved with data analysis, interpretation and drafted the manuscript; Sudhindran S revised the article critically for important intellectual content.

Institutional review board statement: The study was reviewed and approved by the Amrita Institute of Medical sciences Research Review Board (Kochi, Kerala, India).

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrolment.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.



Manuscript source: Invited manuscript

Correspondence to: Rama P Venu, Professor and Head, Department of Gastroenterology, Amrita Institute of Medical Sciences, Ponekkara AIMS PO, Kochi, Kerala 682041, India. ramapvenu@yahoo.com
Telephone: +91-75-61090937

Received: January 28, 2017
Peer-review started: February 14, 2017
First decision: March 7, 2017
Revised: March 23, 2017
Accepted: April 18, 2017
Article in press: April 19, 2017
Published online: May 15, 2017

## Abstract

***AIM***

To identify factors predicting outcome of endoscopic therapy in bile duct strictures (BDS) post living donor liver transplantation (LDLT).

***METHODS***

Patients referred with BDS post LDLT, were retrospectively studied. Patient demographics, symptoms (Pruritus, Jaundice, cholangitis), intra-op variables (cold ischemia time, blood transfusions, number of ducts used, *etc*.), peri-op complications [hepatic artery thrombosis (HAT), bile leak, infections], stricture morphology (length, donor and recipient duct diameters) and relevant laboratory data both pre- and post-endotherapy were studied. Favourable response to endotherapy was defined as symptomatic relief with > 80% reduction in total bilirubin/serum gamma glutamyl transferase. Statistical analysis was performed using SPSS 20.0.

***RESULTS***

Forty-one patients were included (age: 8-63 years). All had right lobe LDLT with duct-to-duct anastomosis. Twenty patients (48.7%) had favourable response to endotherapy. Patients with single duct anastomosis, aggressive stent therapy (multiple endoscopic retrograde

cholagiography, upsizing of stents, dilatation and longer duration of stents) and an initial favourable response to endotherapy were independent predictors of good outcome ($P$ < 0.05). Older donor age, HAT, multiple ductal anastomosis and persistent bile leak (> 4 wk post LT) were found to be significant predictors of poor response on multivariate analysis ($P$ < 0.05).

***CONCLUSION***

Endoscopic therapy with aggressive stent therapy especially in patients with single duct-to-duct anastomosis was associated with a better outcome. Multiple ductal anastomosis, older donor age, shorter duration of stent therapy, early bile leak and HAT were predictors of poor outcome with endotherapy in these patients.

**Key words:** Biliary strictures; Living donor liver transplantation; Endotherapy; Bile leaks



**Core tip:** Living donor liver transplantation (LDLT) is the definitive treatment for end stage liver disease. Biliary complications complicating LDLT is a major source of morbidity and mortality. Anastomotic strictures seen with LDLT are notorious for gross phenotypical variations which have led to variable results with endotherapy as a treatment option in these patients. In this study, we have looked into the factors that can predict the response with endotherapy (endoscopic retrograde cholangiography, sphincterotomy and stent placement) thereby allowing for better prognostication and selection of patients in order to optimize patient care.

Harshavardhan RB, Ahamed H, Panicker S, Sudhindran S, Venu RP. Endoscopic therapy for biliary strictures complicating living donor liver transplantation: Factors predicting better outcome. *World J Gastrointest Pathophysiol* 2017; 8(2): 77-86 Available from: URL: http://www.wjgnet.com/2150-5330/full/v8/i2/77.htm DOI: http://dx.doi.org/10.4291/wjgp.v8.i2.77

## INTRODUCTION

Living donor liver transplantation (LDLT) as a treatment for end stage liver disease is an approach that is becoming increasingly common in Asian centres. Biliary complications, especially bile duct strictures (BDS), remain a significant complication of LDLT[1-4], although there seems to be a decline in incidence over the last 2 decades[5]. Unlike deceased donor liver transplantation (DDLT), the incidence of BDS in patients undergoing LDLT is higher and ranges between 18%-32%[6,7]. Most of these patients present with symptoms of bile duct obstruction such as jaundice, pruritus and/or cholangitis. Endotherapy with endoscopic retrograde cholangiography (ERC), sphincterotomy and placement of endoprosthesis have been shown to be technically feasible and safe for BDS in post LDLT[8,9]. However, therapeutic efficacy for BDS post LDLT has been variable[9-11]. Therefore, additional studies are needed looking into the factors that can predict a favourable therapeutic response to endotherapy.

The higher incidence of BDS post LDLT have been attributed to difficult surgical techniques of anastomosis owing to smaller diameter of the ducts along with multiple ductal anastomoses. The endotherapy protocol (type of stents, sphincterotomy, stricture dilatation, number of ERC with stent exchange and duration of stenting) has not been standardized in earlier reports, possibly accounting for the varying results of endotherapy in these studies[9-15]. The stricture morphology, type and number of stents used, duration of stent therapy may also have a direct bearing on therapeutic outcome. In this study various risk factors like anatomical contortions of the stricture, type and number of ductal anastomosis, duration, diameter as well as number of stents placed and their impact on the clinical as well as biochemical outcomes are carefully analysed.

## MATERIALS AND METHODS

### *Patient population and data collection*

This was a single centre, observational study where all patients referred to the Gastroenterology service with a diagnosis of BDS post LDLT from January 2012 till November 2015 were evaluated. Recipient and donor demographics (age and gender), transplant setting (emergency or elective), aetiology of liver disease, pre-operative Model for End-stage Liver Disease score, intra-operative variables like cold ischemia time, number of blood transfusions, number of bile ducts anastomosed, ductoplasty, conduit used (duct or jejunal loop) and post-operative complications like Hepatic artery thrombosis (HAT), bile leaks; were recorded in pre-designed performas. Bile leaks were considered persistent if the leak did not resolve in four weeks.

### *Endotherapy*

All patients who were medically fit to undergo therapeutic ERC were included in the study. All ERCs were performed by an experienced endoscopist under general anaesthesia and the choice of anaesthetic was left to the discretion of a dedicated anaesthetic doctor who was present throughout the duration for all procedures. A standard adult ERC scope (Olympus TJF Q180V) with an outer diameter of 13.7 mm and channel diameter of 4.2 mm was used. All procedures were performed in the endoscopy suite under fluoroscopic guidance (Fexavision, Shimadzu). Technical success was defined as selective cannulation of CBD with an adequate cholangiogram obtained. Definition of biliary stricture: Patients who had at least two of the three following features were included in the study: (1) Symptoms of cholestasis like Jaundice, pruritus and/or cholangitis in

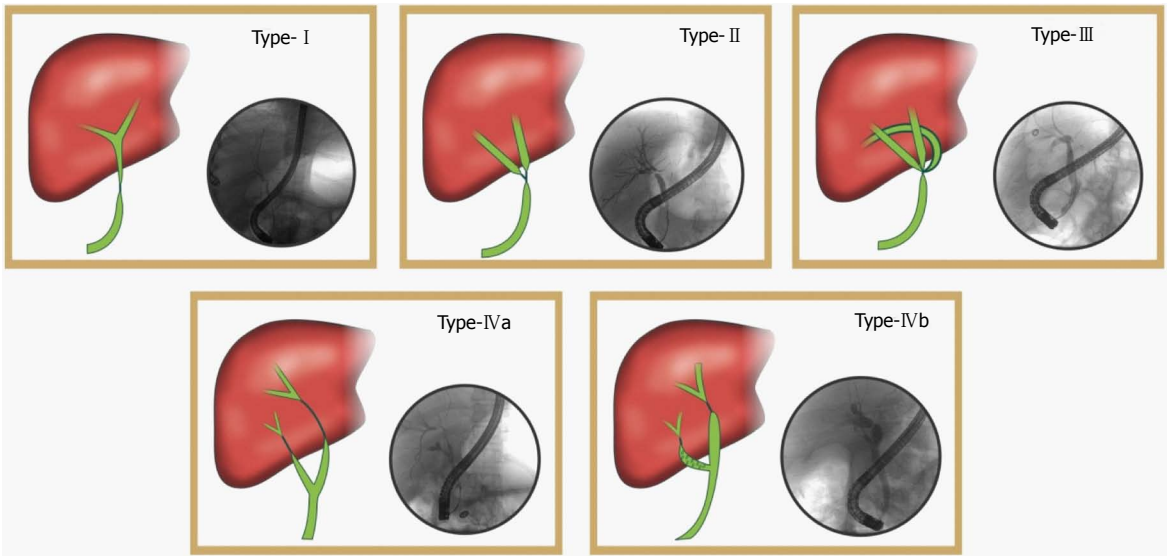


**Figure 1 Classification of anastomotic bile duct strictures post living donor liver transplantation.**

conjunction with elevated serum bilirubin and/or serum gamma glutamyl transferase (GGT) to more than twice the upper limit of normal; (2) Magnetic resonance cholangiopancreatography showing a narrowing with proximal duct dilatation; and (3) ERC cholangiogram showing significant narrowing at the anastomotic site with/without ductal dilatation.

The cholangiograms were then reviewed for stricture morphology (length, diameter, type of anastomoses). The anastomoses were classified as type 1 when single donor duct was anastomosed to a single recipient duct. Type 2 is when two donor ducts are connected to a single recipient duct. Type 3 is when three donor ducts were anastomosed to a single recipient duct. Type 4 is when two recipient ducts (cystic duct and common hepatic duct or right and left hepatic ducts) are anastomosed with the donor duct (Figure 1). The number of ducts anastomosed including the use of cystic duct for anastomoses were also studied.

**Therapeutic protocol and follow up:** Institutional protocol for endoscopic management of benign biliary strictures includes Endoscopic sphincterotomy (5-7 mm), which was performed in all patients and stricture dilation using 4-7 mm balloon dilators were performed. A polyethylene stent of 5-7 Fr diameter and 12-15 cm long was then deployed across the stricture. The stent placement was repeated and the stent graduated to a higher diameter upto 10 Fr, if possible. The procedure was repeated every 3 mo for stent replacement for a minimum period of 1 year. Patients, who did not undergo this optimal stent exchange therapy for 1 year due to non-compliance, were studied for recurrence of symptoms and relevant laboratory studies at subsequent clinic visits.

Patients in whom ERC was unsuccessful (due to tight stricture or complex stricture), a guide wire was advanced into the duodenum across the stricture *via* a percutaneous trans hepatic puncture of the intrahepatic duct (PTBD). ERC, dilatation and stent placement was then carried out using a rendezvous approach. All patients were followed up for immediate and delayed complications.

Surgical repair was undertaken only when both endoscopic and transhepatic approaches failed to cross the stricture, the so called "defiant strictures". Unlike BDS in deceased donors, those occurring in LDLT are surgically formidable to correct, since the length of donor duct (proximal to anastomosis) tends to be very short. BDS following LDLT therefore tend to extend intrahepatically and defining an appropriate donor duct for anastomosis can be a very formidable task surgically particularly in type 4 stictures.

### *Outcome definition*

Outcome was classified into favourable and unfavourable to endotherapy. Patients with favourable response had both biochemical and clinical improvement. Clinical improvement is characterised by relief from symptoms of bile duct obstruction (pruritus, jaundice and cholangitis) and biochemical improvement is considered when there was 80% reduction in total bilirubin (BR)/GGT. Unfavourable response was considered to be partial if either clinical or biochemical improvement was present and Non responsive if neither clinical nor biochemical improvement was seen.

### *Statistical analysis*

Statistical analysis was carried out using IBM SPSS software version 20.0. Comparison of means in the three groups were done using ANOVA test for parametric or Kruskal Wallis for non-parametric variables. Response was also classified as complete responders and partial/No response where independent 2-sample *t* test and

**Table 1 Baseline characteristics of the patient population stratified according to response to endotherapy**

| Parameters | Total ($n$ = 41) | Complete response ($n$ = 20) | Partial response ($n$ = 14) | No response ($n$ = 7) |
|---|---|---|---|---|
| Recipient mean age (year ± SD) | 40.02 ± 15.45 | 37.5 ± 17.03 | 41.0 ± 14.3 | 45.29 ± 13.15 |
| Donor mean age (year ± SD) | 39.9 ± 8.9 | 38.5 ± 6.85 | 39.86 ± 11.2 | 44.0 ± 9.03 |
| Recipient gender - male, $n$ (%) | 36 (87.8) | 16 (80) | 14 (100) | 6 (85.7) |
| Donor gender - male, $n$ (%) | 9 (22) | 4 (20) | 4 (28.5) | 1 (14.2) |
| Transplant setting, $n$ (%) | | | | |
| CLD | 32 (78) | 14 (70) | 12 (85.7) | 6 (85.7) |
| ALF | 9 (22) | 6 (30) | 2 (14.3) | 1 (14.3) |
| Etiology of CLD ($n$ = 32) | | | | |
| Alcohol | 12 (37.5) | 4 (33.3) | 6 (50) | 2 (16.7) |
| Cryptogenic | 13 (40.6) | 6 (46.1) | 5 (38.4) | 2 (15.5) |
| Others (Wilsons, hepatitis B, hepatitis C, autoimmune) | 7 (21.8) | 3 (42.8) | 2 (28.6) | 2 (28.6) |
| MELD | 23.45 ± 6.4 | 25.07 ± 7.2 | 22.46 ± 4.75 | 21.83 ± 6.52 |
| Intra-operative variables | | | | |
| Cold Ischemia time (secs) (mean ±) | 51.5 ± 29.2 | 53.3 ± 34.4 | 54.08 ± 25.6 | 43.57 ± 28.4 |
| Blood transfusions (mean ±) | 4.1 ± 2.8 | 4.3 ± 2.3 | 4.3 ± 3.5 | 3.2 ± 2.2 |
| Post op complications | | | | |
| Hepatic artery thrombosis, $n$ (%) | 5 (12.2) | 0 (0) | 3 (60) | 2 (40) |
| Bile leak | 26 (65) | 12 (60) | 10 (71.4) | 4 (57.1) |
| Mean interval between LT and leak (d) | 18.4 | 20.85 | 12.6 | 5.6 |
| Persistent bile leak > 4 wk, $n$ (%) | 14 (34.1) | 2 (14.2) | 8 (57.1) | 4 (28.7) |
| Clinical presentation, $n$ (%) | | | | |
| Cholangitis | 15 (36.6) | 6 (30) | 7 (42.1) | 2 (13.4) |
| Pruritus alone | 16 (39) | 7 (43.75) | 4 (25) | 5 (31.25) |
| Jaundice | 16 (39) | 5 (31.25) | 6 (37.5) | 5 (31.25) |

CLD: Chronic liver disease; ALF: Acute liver failure; MELD: Model for end-stage liver disease.

Mann Whitney *U* tests was used for parametric and non-parametric variables respectively. Categorical variables were analysed using $\chi^2$ test/Fisher's Exact test in the univariate analysis. Multivariate analysis was then carried out using multiple logistic regression analysis. Kaplan Meier curves were computed to look for a survival advantage. A $P$ value < 0.05 was taken as significant. The statistical methods of this study were reviewed by Mr Unnikrishnan UG, MSc Statistics, Lecturer, Department of Biostatistics, Amrita Institute of Medical Sciences, Kochi, Kerala, India.

## RESULTS

Of the 458 LDLT performed, 47 patients had biliary strictures (10.2%); of which 41 patients had complete data and were included in the study. The mean age of the population was 40.02 ± 15.45 years with a male preponderance (87.8%). Majority of the patients were transplanted in a non-emergency setting (78%) with alcohol (37.5%) and cryptogenic (40.6%) cirrhosis being the most common etiologies for liver disease. The intra-operative variables did not have any association with therapeutic efficacy in this study. However, patients with post-operative HAT and BDS did not have any response to endotherapy despite re-establishment of flow in 4/5 patients. Majority (85.8%) of patients with persistent bile leak > 4 wk had only partial/no improvement to endotherapy ($P$ value < 0.05). Demographic variables and baseline data are shown in Table 1.

### *Technical success and therapeutic efficacy of endotherapy*

ERCP was successful in 31 patients (75.6%) while 10 patients (24.4%) required a combined PTBD with a rendezvous approach. Favourable response to endotherapy was seen in 20 patients (48.7%); 14 patients (34.1%) had partial unfavourable response to endotherapy and 7 patients (17.1%), had no response to endotherapy (Figure 2).

### *Endotherapy in BDS post LDLT*

A total of 117 ERCPs were performed in 41 patients (2.8 procedures per patient) (Table 2). Patients who had complete improvement to endotherapy (favourable response) had a significantly higher number of total ERC (3.01 in patients with favourable response whereas 1.71 in patients with unfavourable response) and the initial favourable response to endotherapy was also higher (85% *vs* 57.1% respectively) ($P$ < 0.05) in these patients. Aggressive stent therapy was performed in 23 patients (56%) and these patients had a significant improvement after endotherapy, 16 patients (69.5%) had complete favourable response while 7 patients (30.5%) had partial/no improvement ($P$ value 0.04). Duration of stent therapy showed a direct impact on therapeutic efficacy where patients with a favourable response to endotherapy having the stent in place for a mean duration of 16.4 mo, whereas, patients who had a partial/no response to endotherapy had the stent in place for a mean duration of 6.8 mo ($P$ value = 0.03)

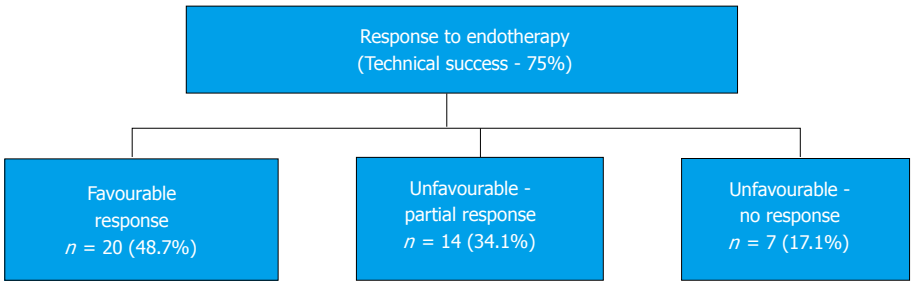


**Figure 2 Therapeutic efficacy of endotherapy in patients with bile duct strictures post living donor liver transplantation.**

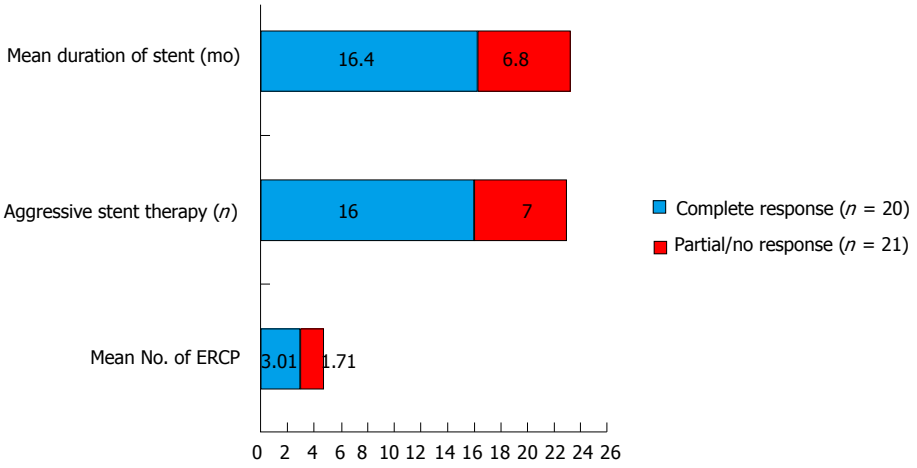


**Figure 3 Statistically significant ($P < 0.05$) correlations of good response to endotherapy.**

**Table 2 Impact of endotherapy protocol and morphology of the stricture on therapeutic efficacy**

| Endotherapy details | Total ($n$ = 41) | Complete response ($n$ = 20) | Partial/no response ($n$ = 21) |
|---|---|---|---|
| Mean time interval between LT and first ERC (d) | | | |
| Mean No. of ERC[a] | 368.3 | 352.6 | 444.1 |
| Mean Stricture length (cm) | 2.85 | 3.01 | 1.71 |
| Initial response to ERC, $n$ (%) | 0.84 | 0.85 | 0.82 |
| Aggressive stent therapy[a,b] | 29 (70.7) | 17 (85) | 12 (57.1) |
| Mean duration of stentinga (mo) | 23 (39) | 16 (69.5) | 7 (23.3) |
| Morphology of the stricture | 5.75 | 16.4 | 6.8 |
| Mean length of stricture (mm) | 84 | 82 | 85 |
| Type of anastomosis (Figure 1), $n$ (%) | | | |
| Type I | 13 | 10 (76.9) | 3 (23.1) |
| Type II | 16 | 9 (56.25) | 7 (43.75) |
| Type III | 5 | 1 (20) | 4 (80) |
| Type IV | 7 | 0 (14.2) | 7 (100) |

[a]$P$ value < 0.05; [b]Aggressive stent therapy involves sphincterotomy, serial dilatation of stricture and increase in number/size of stents. LT: Liver transplantation; ERC: Endoscopic retrograde cholangiography.

(Figure 3). A minimum duration of 6.75 mo of stent therapy was found to be predictive of a favourable response to endotherapy (AUROC 0.75).

**Stricture morphology:** Stricture length and duct diameters did not have a significant correlation to eventual response to endotherapy. The type of anastomosis, however (Figure 1), did influence the response to endotherapy. All patients with type Ⅳ (2 recipient ducts) anastomosis had a poor response to endotherapy (Table 2).

Surgical repair was performed only for one patient in this cohort, who remains well following surgery. In one other patient, who developed secondary biliary cirrhosis, retransplantation was required.

### *Safety and complication*

Endotherapy in this patient cohort was safe and only 1 patient had mild acute pancreatitis and 1 patient had cholangitis. A total of 9 patients died (21.9%), including the one with cholangitis. However, a statistically significant survival advantage was observed in patients

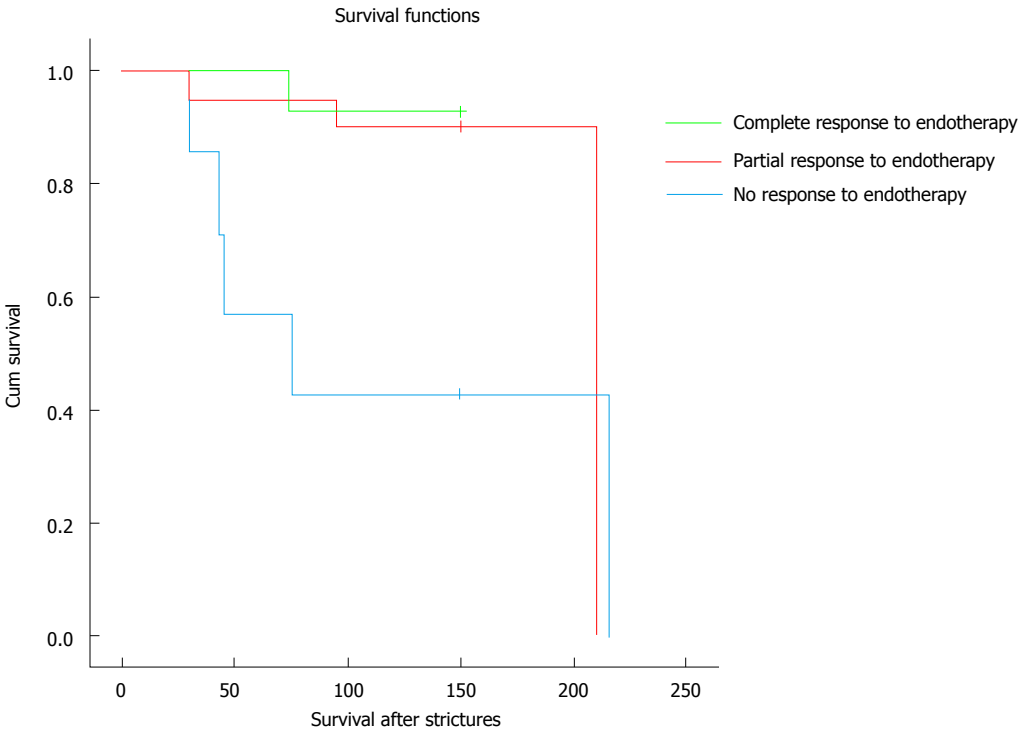


**Figure 4 Kaplan meier curves showing a clear survival benefit in patients who had a response to endotherapy.**

who had a favourable response to endotherapy as opposed to patients who did not (*P* value 0.02) (Figure 4).

## DISCUSSION

Liver transplantation remains the only definitive treatment for end stage liver disease. Transplantation using living donors, which is commonly practised in most Asian centres including India, is fraught with unique technical challenges and specific complications with as yet, evolving avenues of therapy. Biliary complications (BDS and Bile leaks) in particular, continue to be a stumbling block resulting in considerable morbidity and mortality among these patients[2,16-18]. The incidence of BDS is higher in LDLT patients (10%-30%) compared to DDLT possibly related to smaller diameter of the ducts and multiple ductal anastomoses[19,20].

BDS in the post-transplant setting is classified into one of two types - anastomotic (AS) and non-anastomotic (NAS) strictures. NAS, more common with DDLT, with an incidence of 5%-15%[17,18] are longer, multiple and located proximal to the anastomotic site. NAS are further divided into microangiopathic (with prolonged cold ischemia times), macroangiopathic (in association with HAT) and immunogenic (in association with rejection). AS on the other hand, are seen more frequently in patients who have received living donor livers and tend to be shorter, confined to the anastomotic site and present later in the course of follow up[5]. Multiple factors contribute to the development of AS including surgical technique, number of ducts anastomosed and ischemia[21]. The complexity of the microvasculature of the bile ducts makes them particularly vulnerable to ischemia. Management of biliary strictures with endotherapy, *i.e.*, ERC with endobiliary stenting, is the treatment of choice especially for patients with AS[22]. They are notoriously difficult to treat with a recurrence rate between 9%-37%[23]. However, contrary to this, a report from the Adult-to-Adult Living Donor Liver Transplantation Cohort Study consortium (A2ALL)[24] suggested a comparable efficacy of endoscopic treatment in anastomotic strictures (in both DDLT as well as LDLT setting) based on which a recommendation to reserve endoscopic therapy as a first line treatment for all patients with anastomotic strictures could be made[22]. Unfortunately, some of these patients may not have a favourable response to endotherapy resulting in significant morbidity and even mortality. These patients with the so-called "defiant" biliary strictures may end up with re-surgery or repeat transplantation which will add to the financial burden of the patients[22].

Several investigators have reported a favourable response following endotherapy in the range of 53%-88%[3,19,25,26]. In our patient population, a favourable response was seen in only 48.7% of patients, while partial relief(clinical or biochemical) was noted in 34.1% of patients. Seven/41 patients (17.1%) in our study had no response to endotherapy. Moreover patients who did not respond to endotherapy were at a survival disadvantage with high mortality rate. This critical observation provided us with the impetus to study the factors that might predict outcome with endotherapy, especially

since there is a considerable variation of results and lack of uniform treatment protocols.

Donor age was found to have a significant bearing on response to endotherapy. The age related variations in duct calibre and fibrotic response to ischemia/inflammation of the ducts may be possible pathogenic factors for the development of complex BDS. HAT in the post-operative period was a significant predictor of poor response to endotherapy despite re-establishment of flow in most of the patients (4/5 patients, 80%). This suggests that the ischemic stress even though "short-lived" was sufficient to significantly compromise the viablity of the anastomotic site leading to complex and defiant strictures.

Bile leaks have previously been shown to be associated with an increased incidence of BDS[27-29]. Inflammatory reactions due to the extravasated bile leading to fibrosis has been proposed as the pathogenetic mechanism in these patients. Alternatively, bile leak may be a surrogate to indicate ischemia and necro inflammatory reaction which eventually results in BDS[30]. Our study looked into not just the immediate perioperative bile leak but also the duration of leak, of more than 4 wk. Patients who had persistent bile leak more than four weeks had a poor outcome following endotherapy which may in part, be related to a dose response relationship between persistent inflammation caused by the extravasated bile. As a corollary to this, an early and aggressive treatment protocol with endoscopic sphincterotomy and stent placement may positively impact endoscopic outcome for BDS in these patients.

LDLT strictures are notoriously complex possibly resulting from multiple ductal anastomoses, mismatch of duct sizes and altered anatomy[31]. Only 75% technical success rate was achieved with ERC in our patient population with 10 patients requiring PTBD followed by rendezvous approach for stent placement. In all these patients the strictures assumed complex anatomical contortions.

Multiple ductal anastomoses are seldom considered as a risk factor for BDS post LDLT[32]. In a study by Chan *et al*[33], they found that a single hepatic duct is ideal for a duct to duct anastomosis. If two ducts are present, a duct to duct anastomosis can still be performed provided the ducts are less than 3 mm apart. If the ducts are more than 3 mm apart, they suggested separate Hepaticojejunostomies using a Roux-en-T loop[33]. Another study by Asonuma *et al*[34] explored the feasibility of the use of the recipient cystic duct for biliary reconstruction in right liver donor transplantation when two bile duct orifices were present. Our study also revealed that employing multiple recipient ducts for anastomosis can cause defiant and complex strictures resistant to endotherapy. Moreover when recipient cystic duct was used for anastomosis, the outcome for endotherapy was especially poor. The stricture length did not correlate with response to endotherapy suggesting a complex anatomical spatial alteration as the likely cause for poor response to endotherapy than longitudinal extent of the stricture.

There is no uniform consensus on the ERC protocol for the treatment of post LDLT BDS. Several reports have suggested that balloon dilatation with endobiliary stenting is superior to dilatation alone[17,35-37]. However, in a meta-analysis published recently, balloon dilatation with stenting did not afford a significant advantage to dilatation alone[38]. Current practices for management of BDS post LDLT suggests a 3 mo interval for repeat procedures with stricture dilatation and stent exchanges, which has been shown to minimize stent occlusion and prevent cholangitis or stone formation[17,22,39]. Our results also indicate that an aggressive endotherapy protocol which included stent exchanges every 3 mo, stent up-sizing to a maximum of 10 Fr, serial dilatation of the strictures and prolonged duration of stent therapy were associated with a favourable response to endotherapy. Similar to our experience Verdonk *et al*[30] had a 75% success rate for post LDLT strictures with a median of 3 endotherapy sessions per patient. They also showed that higher number of endotherapy sessions with increasing number of stents had a good outcome. A predictive cut-off of 6.75 mo for the duration of stent therapy was found to have reasonable validity for eventual favourable response to endotherapy. Most studies in this area have found an average stent duration between 6 mo to 1 year with 3-4 endotherapy sessions to be adequate in preventing recurrences with minimal complications and morbidity[7,40,41].

Reported complications for endotherapy in BDS post transplantation include post-ERC pancreatitis, cholangitis, bleeding, pain and stent migration. In this study, ERC followed by endotherapy was found to be safe with only one patient developing mild acute pancreatitis and one developing cholangitis. Premature removal of stents without replacement or up-sizing of stent was associated with recurrent bile duct stricture and cholestatic jaundice.

The treatment of "defiant" BDS remains an elusive area of study where innovative endoscopic approaches like the use of digital single operator cholangioscopy (DSOC)[42], covered self-expanding metallic stent (c-SEMS)[43], use of multiple plastic stents and novel dilatation balloons[22] are steadily gaining ground over traditional eventual surgical options which include hepaticojejunostomy and retransplantation[22,44]. In this study, 2 patients with "defiant" BDS underwent surgery [Hepaticojejunostomy (1) and Retransplantation (1)] and one patient had a c-SEMS placed. The use of c-SEMS has recently been studied in a meta-analysis[38] where it was shown to have superior efficacy rates as compared to multiple plastic stenting. However, the same analysis showed high complication rates, especially stent migration[38]. In our study, 2 patients with difficult BDS underwent DSOC with the help of which we were able to traverse the stricture successfully only in 1 patient. Our approach to "defiant" BDS currently centres around the

use of multiple plastic stents with only selected patients receiving c-SEMS. Surgical correction is reserved for patients who have particularly crippling symptoms along with frequent recurrence of strictures despite maximal stent therapy for over a period of 15 mo.

In summary, BDS remains a major complication of LDLT. Early identification of risk factors such as bile leaks and HAT in conjunction with adopting early treatment measures may improve therapeutic efficacy of endotherapy in these patients. Aggressive endotherapy protocols seem to have a favourable outcome after endotherapy thereby reducing the associated morbidity and mortality of BDS post LDLT. The use of c-SEMS, DSOC in the management of "defiant" BDS is an area that requires further study.

## COMMENTS

### Background

Bile duct strictures (BDS) are a major cause of morbidity and mortality in patients who have undergone a living donor liver transplantation. Endotherapy for BDS in these patients includes endoscopic retrograde cholangiography, sphincterotomy and stent placement. Results of endotherapy are variable and factors predicting outcome are not well elucidated.

### Research frontiers

Factors that can predict endotherapy outcome and result in optimal patient selection. Endotherapy protocols/guidelines which can help roadmap this heterogenous group of patients towards better healthcare.

### Innovations and breakthroughs

Unique outcome definition: The outcome of endotherapy was considered favourable only when there was symptomatic and biochemical improvement. Partial responders were considered unfavourable owing to significant morbidity in this group. This sub group of patients is particulary tricky as they find themselves in a therapeutic "no man's land" so to speak, where established treatment has provided some improvement, but with residual yet significant morbidity. Aggressive endotherapy protocols with repeated procedures, serial dilatation and stent upsizing for a minimum period exceeding 6 mo can yield good outcomes in majority of patients. Early endotherapy in patients with persistent bile leak (> 4 wk) may prevent the development of complex BDS. Use of two or more recipient ducts, especially cystic ducts are particularly poor responders to endotherapy.

### Applications

This study may aid in the establishment of uniform endotherapy protocols which can have a wide applicability beyond the confines of single centre experiences. Bile duct anastomosis using multiple recipient ducts may be avoided, if possible; in favour of innovative surgical practices that may impact overall outcome in these patients. The outcome definition which identifies partial responders may suggest a sub group that needs a more detailed assessment which can lend clarity on optimal management of these patients.

### Terminology

Type of anastomosis has been classified in terms of number of anastomosis into 4 types which have a significant bearing on overall outcome. Outcome definition was defined as favourable (symptomatic and biochemical improvement) and unfavourable (partial/non responders). Partial responders are those with either symptomatic and biochemical improvement and non-responders were labelled as those with neither.

### Peer-review

The manuscript focuses on the endoscopic treatment of biliary strictures in living donor liver transplantation. It is interesting and well written.

## REFERENCES


1 **Wang SF**, Huang ZY, Chen XP. Biliary complications after living donor liver transplantation. *Liver Transpl* 2011; **17**: 1127-1136 [PMID: 21761548 DOI: 10.1002/lt.22381]

2 **Dulundu E**, Sugawara Y, Sano K, Kishi Y, Akamatsu N, Kaneko J, Imamura H, Kokudo N, Makuuchi M. Duct-to-duct biliary reconstruction in adult living-donor liver transplantation. *Transplantation* 2004; **78**: 574-579 [PMID: 15446317 DOI: 10.1097/01.TP.0000128912.09581.46]

3 **Gondolesi GE**, Varotti G, Florman SS, Muñoz L, Fishbein TM, Emre SH, Schwartz ME, Miller C. Biliary complications in 96 consecutive right lobe living donor transplant recipients. *Transplantation* 2004; **77**: 1842-1848 [PMID: 15223901 DOI: 10.1097/01.TP.0000123077.78702.0C]

4 **Ramacciato G**, Varotti G, Quintini C, Masetti M, Di Benedetto F, Grazi GL, Ercolani G, Cescon M, Ravaioli M, Lauro A, Pinna A. Impact of biliary complications in right lobe living donor liver transplantation. *Transpl Int* 2006; **19**: 122-127 [PMID: 16441361 DOI: 10.1111/j.1432-2277.2005.00248.x]

5 **Simoes P**, Kesar V, Ahmad J. Spectrum of biliary complications following live donor liver transplantation. *World J Hepatol* 2015; **7**: 1856-1865 [PMID: 26207167 DOI: 10.4254/wjh.v7.i14.1856]

6 **Gómez CM**, Dumonceau JM, Marcolongo M, de Santibañes E, Ciardullo M, Pekolj J, Palavecino M, Gadano A, Dávolos J. Endoscopic management of biliary complications after adult living-donor versus deceased-donor liver transplantation. *Transplantation* 2009; **88**: 1280-1285 [PMID: 19996927 DOI: 10.1097/TP.0b013e3181bb48c2]

7 **Thuluvath PJ**, Pfau PR, Kimmey MB, Ginsberg GG. Biliary complications after liver transplantation: the role of endoscopy. *Endoscopy* 2005; **37**: 857-863 [PMID: 16116539 DOI: 10.1055/s-2005-870192]

8 **Chok KS**, Chan SC, Cheung TT, Sharr WW, Chan AC, Fan ST, Lo CM. A retrospective study on risk factors associated with failed endoscopic treatment of biliary anastomotic stricture after right-lobe living donor liver transplantation with duct-to-duct anastomosis. *Ann Surg* 2014; **259**: 767-772 [PMID: 23657086 DOI: 10.1097/SLA.0b013e318294d0ce]

9 **Tsujino T**, Isayama H, Sugawara Y, Sasaki T, Kogure H, Nakai Y, Yamamoto N, Sasahira N, Yamashiki N, Tada M, Yoshida H, Kokudo N, Kawabe T, Makuuchi M, Omata M. Endoscopic management of biliary complications after adult living donor liver transplantation. *Am J Gastroenterol* 2006; **101**: 2230-2236 [PMID: 16952286 DOI: 10.1111/j.1572-0241.2006.00797.x]

10 **Kato H**, Kawamoto H, Tsutsumi K, Harada R, Fujii M, Hirao K, Kurihara N, Mizuno O, Ishida E, Ogawa T, Fukatsu H, Yamamoto K, Yagi T. Long-term outcomes of endoscopic management for biliary strictures after living donor liver transplantation with duct-to-duct reconstruction. *Transpl Int* 2009; **22**: 914-921 [PMID: 19497069 DOI: 10.1111/j.1432-2277.2009.00895.x]

11 **Kurita A**, Kodama Y, Minami R, Sakuma Y, Kuriyama K, Tanabe W, Ohta Y, Maruno T, Shiokawa M, Sawai Y, Uza N, Yazumi S, Yoshizawa A, Uemoto S, Chiba T. Endoscopic stent placement above the intact sphincter of Oddi for biliary strictures after living donor liver transplantation. *J Gastroenterol* 2013; **48**: 1097-1104 [PMID: 23325164 DOI: 10.1007/s00535-012-0705-x]

12 **Lee YY**, Gwak GY, Lee KH, Lee JK, Lee KT, Kwon CH, Joh JW, Lee SK. Predictors of the feasibility of primary endoscopic management of biliary strictures after adult living donor liver transplantation. *Liver Transpl* 2011; **17**: 1467-1473 [PMID: 21898773 DOI: 10.1002/lt.22432]

13 **Chang JH**, Lee IS, Choi JY, Yoon SK, Kim DG, You YK, Chun HJ, Lee DK, Choi MG, Chung IS. Biliary Stricture after Adult Right-Lobe Living-Donor Liver Transplantation with Duct-to-Duct Anastomosis: Long-Term Outcome and Its Related Factors after Endoscopic Treatment. *Gut Liver* 2010; **4**: 226-233 [PMID: 20559526 DOI: 10.5009/gnl.2010.4.2.226]

14 **Kim ES**, Lee BJ, Won JY, Choi JY, Lee DK. Percutaneous transhepatic biliary drainage may serve as a successful rescue

procedure in failed cases of endoscopic therapy for a post-living donor liver transplantation biliary stricture. *Gastrointest Endosc* 2009; **69**: 38-46 [PMID: 18635177 DOI: 10.1016/j.gie.2008.03.1113]

15 **Kim TH**, Lee SK, Han JH, Park DH, Lee SS, Seo DW, Kim MH, Song GW, Ha TY, Kim KH, Hwang S, Lee SG. The role of endoscopic retrograde cholangiography for biliary stricture after adult living donor liver transplantation: technical aspect and outcome. *Scand J Gastroenterol* 2011; **46**: 188-196 [PMID: 20955089 DOI: 10.3109/00365521.2010.522722]

16 **Duffy JP**, Kao K, Ko CY, Farmer DG, McDiarmid SV, Hong JC, Venick RS, Feist S, Goldstein L, Saab S, Hiatt JR, Busuttil RW. Long-term patient outcome and quality of life after liver transplantation: analysis of 20-year survivors. *Ann Surg* 2010; **252**: 652-661 [PMID: 20881772 DOI: 10.1097/sla.0b013e3181f5f23a]

17 **Sharma S**, Gurakar A, Jabbour N. Biliary strictures following liver transplantation: past, present and preventive strategies. *Liver Transpl* 2008; **14**: 759-769 [PMID: 18508368 DOI: 10.1002/lt.21509]

18 **Graziadei IW**, Schwaighofer H, Koch R, Nachbaur K, Koenigsrainer A, Margreiter R, Vogel W. Long-term outcome of endoscopic treatment of biliary strictures after liver transplantation. *Liver Transpl* 2006; **12**: 718-725 [PMID: 16482553 DOI: 10.1002/lt.20644]

19 **Ribeiro JB**, Martins Fde S, Garcia JH, Cunha AC, Pinto RA, Satacaso MV, Prado-Júnior FP, Pessoa RR. Endoscopic management of biliary complications after liver transplantation. *Arq Bras Cir Dig* 2012; **25**: 269-272 [PMID: 23411927 DOI: 10.1590/S0102-67202012000400011]

20 **Lee DW**, Jo HH, Abdullah J, Kahaleh M. Endoscopic Management of Anastomotic Strictures after Liver Transplantation. *Clin Endosc* 2016; **49**: 457-461 [PMID: 27744664 DOI: 10.5946/ce.2016.130]

21 **Seehofer D**, Eurich D, Veltzke-Schlieker W, Neuhaus P. Biliary complications after liver transplantation: old problems and new challenges. *Am J Transplant* 2013; **13**: 253-265 [PMID: 23331505 DOI: 10.1111/ajt.12034]

22 **Shin M**, Joh JW. Advances in endoscopic management of biliary complications after living donor liver transplantation: Comprehensive review of the literature. *World J Gastroenterol* 2016; **22**: 6173-6191 [PMID: 27468208 DOI: 10.3748/wjg.v22.i27.6173]

23 **Koh PS**, Chan SC. Adult-to-adult living donor liver transplantation: Operative techniques to optimize the recipient's outcome. *J Nat Sci Biol Med* 2017; **8**: 4-10 [PMID: 28250667 DOI: 10.4103/0976-9668.198356]

24 **Zimmerman MA**, Baker T, Goodrich NP, Freise C, Hong JC, Kumer S, Abt P, Cotterell AH, Samstein B, Everhart JE, Merion RM. Development, management, and resolution of biliary complications after living and deceased donor liver transplantation: a report from the adult-to-adult living donor liver transplantation cohort study consortium. *Liver Transpl* 2013; **19**: 259-267 [PMID: 23495079 DOI: 10.1002/lt.23595]

25 **Wadhawan M**, Kumar A, Gupta S, Goyal N, Shandil R, Taneja S, Sibal A. Post-transplant biliary complications: an analysis from a predominantly living donor liver transplant center. *J Gastroenterol Hepatol* 2013; **28**: 1056-1060 [PMID: 23432435 DOI: 10.1111/jgh.12169]

26 **Yazumi S**, Yoshimoto T, Hisatsune H, Hasegawa K, Kida M, Tada S, Uenoyama Y, Yamauchi J, Shio S, Kasahara M, Ogawa K, Egawa H, Tanaka K, Chiba T. Endoscopic treatment of biliary complications after right-lobe living-donor liver transplantation with duct-to-duct biliary anastomosis. *J Hepatobiliary Pancreat Surg* 2006; **13**: 502-510 [PMID: 17139423 DOI: 10.1007/s00534-005-1084-y]

27 **Ostroff JW**. Post-transplant biliary problems. *Gastrointest Endosc Clin N Am* 2001; **11**: 163-183 [PMID: 11175980]

28 **Bourgeois N**, Deviére J, Yeaton P, Bourgeois F, Adler M, Van De Stadt J, Gelin M, Cremer M. Diagnostic and therapeutic endoscopic retrograde cholangiography after liver transplantation. *Gastrointest Endosc* 1995; **42**: 527-534 [PMID: 8674922 DOI: 10.1016/s0016-5107(95)70005-6]

29 **Krok KL**, Cárdenas A, Thuluvath PJ. Endoscopic management of biliary complications after liver transplantation. *Clin Liver Dis* 2010; **14**: 359-371 [PMID: 20682241 DOI: 10.1016/j.cld.2010.03.008]

30 **Verdonk RC**, Buis CI, Porte RJ, van der Jagt EJ, Limburg AJ, van den Berg AP, Slooff MJ, Peeters PM, de Jong KP, Kleibeuker JH, Haagsma EB. Anastomotic biliary strictures after liver transplantation: causes and consequences. *Liver Transpl* 2006; **12**: 726-735 [PMID: 16628689 DOI: 10.1002/lt.20714]

31 **Freise CE**, Gillespie BW, Koffron AJ, Lok AS, Pruett TL, Emond JC, Fair JH, Fisher RA, Olthoff KM, Trotter JF, Ghobrial RM, Everhart JE. Recipient morbidity after living and deceased donor liver transplantation: findings from the A2ALL Retrospective Cohort Study. *Am J Transplant* 2008; **8**: 2569-2579 [PMID: 18976306 DOI: 10.1111/j.1600-6143.2008.02440.x]

32 **Salvalaggio PR**, Whitington PF, Alonso EM, Superina RA. Presence of multiple bile ducts in the liver graft increases the incidence of biliary complications in pediatric liver transplantation. *Liver Transpl* 2005; **11**: 161-166 [PMID: 15666393 DOI: 10.1002/lt.20288]

33 **Chan SC**, Fan ST. Biliary complications in liver transplantation. *Hepatol Int* 2008; **2**: 399-404 [PMID: 19669315 DOI: 10.1007/s12072-008-9092-z]

34 **Asonuma K**, Okajima H, Ueno M, Takeichi T, Zeledon Ramirez ME, Inomata Y. Feasibility of using the cystic duct for biliary reconstruction in right-lobe living donor liver transplantation. *Liver Transpl* 2005; **11**: 1431-1434 [PMID: 16237710 DOI: 10.1002/lt.20496]

35 **Zoepf T**, Maldonado-Lopez EJ, Hilgard P, Malago M, Broelsch CE, Treichel U, Gerken G. Balloon dilatation vs. balloon dilatation plus bile duct endoprostheses for treatment of anastomotic biliary strictures after liver transplantation. *Liver Transpl* 2006; **12**: 88-94 [PMID: 16382450 DOI: 10.1002/lt.20548]

36 **Morelli J**, Mulcahy HE, Willner IR, Cunningham JT, Draganov P. Long-term outcomes for patients with post-liver transplant anastomotic biliary strictures treated by endoscopic stent placement. *Gastrointest Endosc* 2003; **58**: 374-379 [PMID: 14528211 DOI: 10.1067/S0016-5107(03)00011-7]

37 **Park JS**, Kim MH, Lee SK, Seo DW, Lee SS, Han J, Min YI, Hwang S, Park KM, Lee YJ, Lee SG, Sung KB. Efficacy of endoscopic and percutaneous treatments for biliary complications after cadaveric and living donor liver transplantation. *Gastrointest Endosc* 2003; **57**: 78-85 [PMID: 12518136 DOI: 10.1067/mge.2003.11]

38 **Aparicio DPDS**, Otoch JP, Montero EFDS, Khan MA, Artifon ELDA. Endoscopic approach for management of biliary strictures in liver transplant recipients: A systematic review and meta-analysis. *United Eur Gastroent* 2016; **0**: 1-19 [DOI: 10.1177/2050640616681909]

39 **Buxbaum JL**, Biggins SW, Bagatelos KC, Ostroff JW. Predictors of endoscopic treatment outcomes in the management of biliary problems after liver transplantation at a high-volume academic center. *Gastrointest Endosc* 2011; **73**: 37-44 [PMID: 21074761 DOI: 10.1016/j.gie.2010.09.007]

40 **Balderramo D**, Navasa M, Cardenas A. Current management of biliary complications after liver transplantation: emphasis on endoscopic therapy. *Gastroenterol Hepatol* 2011; **34**: 107-115 [PMID: 20692731 DOI: 10.1016/j.gastrohep.2010.05.008]

41 **Venu M**, Brown RD, Lepe R, Berkes J, Cotler SJ, Benedetti E, Testa G, Venu RP. Laboratory diagnosis and nonoperative management of biliary complications in living donor liver transplant patients. *J Clin Gastroenterol* 2007; **41**: 501-506 [PMID: 17450034 DOI: 10.1097/01.mcg.0000247986.95053.2a]

42 **Balderramo D**, Sendino O, Miquel R, de Miguel CR, Bordas JM, Martinez-Palli G, Leoz ML, Rimola A, Navasa M, Llach J, Cardenas A. Prospective evaluation of single-operator peroral cholangioscopy in liver transplant recipients requiring an evaluation of the biliary tract. *Liver Transpl* 2013; **19**: 199-206 [PMID: 23404861 DOI: 10.1002/lt.23585]

43 **Kaffes A**, Griffin S, Vaughan R, James M, Chua T, Tee H, Dinesen L, Corte C, Gill R. A randomized trial of a fully covered self-expandable metallic stent versus plastic stents in anastomotic biliary strictures after liver transplantation. *Therap Adv Gastroenterol* 2014; **7**: 64-71 [PMID: 24587819 DOI: 10.1177/1756283X13503614]

44 **Kao D**, Zepeda-Gomez S, Tandon P, Bain VG. Managing the post-liver transplantation anastomotic biliary stricture: multiple plastic versus metal stents: a systematic review. *Gastrointest Endosc* 2013; **77**: 679-691 [PMID: 23473000 DOI: 10.1016/j.gie.2013.01.015]

**P- Reviewer**: De Carlis R, Kim SM, Wejman J **S- Editor**: Ji FF
**L- Editor**: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pathophysiology

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

*World J Gastrointest Pathophysiol* 2017 May 15; 8(2): 87-92

DOI: 10.4291/wjgp.v8.i2.87

ISSN 2150-5330 (online)

CASE REPORT

# Differential diagnosis in ulcerative colitis in an adolescent: Chronic granulomatous disease needs extra attention

Daniel Kotlarz, Odul Egritas Gurkan, Zehra Sule Haskologlu, Ozgur Ekinci, Aysel Aksu Unlusoy, Neslihan Gürcan Kaya, Jacek Puchalka [Deceased], Cristoph Klein, Buket Dalgic

Daniel Kotlarz, Jacek Puchalka, Cristoph Klein, Department of Pediatrics Dr. Von Hauner Children's Hospital, Ludwig-Maximilians-University, 80539 Munich, Germany

Odul Egritas Gurkan, Aysel Aksu Unlusoy, Neslihan Gürcan Kaya, Buket Dalgic, Department of Pediatric Gastroenterology, Gazi University School of Medicine, 06510 Ankara, Turkey

Zehra Sule Haskologlu, Department of Pediatric Immunology and Allergy, Ankara University School of Medicine, 06510 Ankara, Turkey

Ozgur Ekinci, Department of Pathology, Gazi University Faculty of Medicine, 06510 Ankara, Turkey

Author contributions: All authors contributed to this manuscript.

Institutional review board statement: This case report was exempt from the Institutional Review Board standards at Gazi University in Ankara.

Informed consent statement: Informed consent of the subject is taken.

Conflict-of-interest statement: None to declare.



Manuscript source: Invited manuscript

Correspondence to: Odul Egritas Gurkan, MD, Department of Pediatric Gastroenterology, Gazi University School of Medicine, Mevlana Boulevard, Besevler, 06510 Ankara, Turkey. odulmd2003@yahoo.com
Telephone: +90-31-22024423
Fax: +90-31-22024423

Received: November 22, 2016
Peer-review started: November 24, 2016
First decision: December 15, 2016
Revised: March 11, 2017
Accepted: April 6, 2017
Article in press: April 10, 2017
Published online: May 15, 2017

## Abstract

Chronic granulomatous disease (CGD) is a primary immune deficiency that is commonly diagnosed under the age of 5 years (95%) and is rarely seen in adulthood. CGD may manifest as inflammatory bowel disease (IBD) in childhood. Without proper diagnosis, these patients may be monitored for years as IBD; some may even be regarded as steroid-resistant ulcerative colitis (UC) and end up having a colectomy. In this case report, we described a patient who had been followed-up for years as UC and subsequently underwent colectomy, but was finally diagnosed in adulthood as primary immune deficiency.

**Key words:** Ulcerative colitis; Chronic granulomatous disease; Inflammatory bowel disease; Immunodeficiency; Childhood



**Core tip:** In the context of current knowledge, monogenetic diseases are at the basis of early-onset inflammatory bowel disease (IBD). Genetic and immunological studies on this subject have gained momentum in recent years. Chronic granulomatous disease (CGD) is an immune deficiency and may manifest itself as IBD. In an adolescent patient, CGD may present with clinical

signs of IBD without evidence of immune deficiency. It should be kept in mind that CGD in adolescents may extend to adulthood.

Kotlarz D, Egritas Gurkan O, Haskologlu ZS, Ekinci O, Aksu Unlusoy A, Gürcan Kaya N, Puchalka J, Klein C, Dalgic B. Differential diagnosis in ulcerative colitis in an adolescent: Chronic granulomatous disease needs extra attention. *World J Gastrointest Pathophysiol* 2017; 8(2): 87-92 Available from: URL: http://www.wjgnet.com/2150-5330/full/v8/i2/87.htm DOI: http://dx.doi.org/10.4291/wjgp.v8.i2.87

## INTRODUCTION

Chronic granulomatous disease (CGD) is a primary immunodeficiency seen in approximately 1 in 200-250000 individuals[1]. CGD is caused by abnormalities in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex resulting in defective production of reactive oxygen species by phagocytic cells. The NADPH oxidase enzyme complex consists of at least five subunits: gp91phox, p22phox, p47phox, p67phox, and p40phox. Approximately, 66% of all CGD cases result from mutations within the X-linked [*CYBB* gene (encoding gp91phox)], followed by the autosomal recessive forms of CGD (%30) with defects in the (*NCF1*) gene coding for p47phox. Only 5% of the cases are due to mutations in CYBA, NCF-2 or NCF-4 which encode for p22phox, p67phox and p40phox respectively[2,3].

As a result of the failure to activate the respiratory burst, patients with CGD present with severe recurrent intracellular bacterial and fungal infections such as pneumonia, lymphadenitis, cutaneous and hepatic abscesses, osteomyelitis and septicemia[3]. These severe infections are caused predominantly by *Staphylococcus aureus* Aspergillus species, Candida, enteric gram negative bacteria, *Serratia marcescens*, *Burkholderia cepacia* complex, or *Mycobacterium tuberculosis*. CGD patients may also present with diffuse granulomas that can become large enough to cause obstructive or painful symptoms in the esophagus, stomach, biliary, intestinal, urogenital, or pulmonary systems[1-3].

Colitis is an important gastrointestinal manifestation of CGD, typically seen more commonly, but not exclusively in the X-linked form of the disease[4]. The clinical presentation of colitis is similar to that of inflammatory bowel disease (IBD), either ulcerative colitis (UC) or Crohn's disease, and includes diarrhea, abdominal pain and rectal bleeding. A diagnosis of CGD is usually made by gastroenterologists who have previously labeled patients as therapy-resistant IBD[1-3].

## CASE REPORT

A 15-year-old male patient was admitted to a medical center with persistent mucoid, bloody diarrhea. A diagnosis of UC had been made and the patient was followed for six months after discharge. In the interim, treatment comprised pulsed steroids, 5-aminosalicylic acid, and azathioprine. The patient was referred to our center due to persisted symptoms. Medical history revealed absence of recurrent bacterial and fungal infections or frequent antibiotic use. His parents were non-consanguineous.

On physical examination body weight was 51 kg (25$^{th}$ percentile), and the height was 156 cm (10$^{th}$ percentile). Systemic examination was normal. Perianal modifier was not observed. Laboratory results showed hemoglobin of 11.4 g/dL, white blood cell count of 8900/mm$^3$, neutrophils count of 7900/mm$^3$, lymphocyte count of 2200/mm$^3$ and platelet count of 448000/mm$^3$. Stool microscopy showed the presence of occult blood and leukocytes. Total protein was 6.2 g/dL, albumin was 3.8 g/dL, erythrocyte sedimentation rate was 68 mm/h, C-reactive protein was 38 mg/dL, and PANCA was positive. IgA: 68 mg/dL, IgG: 1100 mg/dL, IgM: 79.7 mg/dL, IgE: 38.5 mg/dL, IgA: 217 mg/dL, total C3: 128 mg/dL, total C4: 26.3 mg/dL. Liver and kidney function tests were normal. Colonoscopy was compatible with the diagnosis of pancolitis, but the terminal ileum was normal. Upper gastrointestinal endoscopy revealed pangastritis. Histopathologic evaluation showed cryptitis, crypt abscesses, and pseudopolyps of the entire colonic mucosa; granulomas were not detected.

The subsequent medical treatment and follow-up of the patient are shown in Figure 1. Due to the refractory course of the patient, familial Mediterranean fever (MEVF mutation) and Behcet's disease (Pathergy test, HLA B5 test) were considered and excluded. The patient had a steroid-resistant course and total colectomy was performed. Light microscopic examination of colectomy specimen revealed crypt distortion, cryptitis, crypt loss and crypt abscesses throughout whole colonic mucosa. Epithelial ulcerations, regenerative changes and pseudopolyps were also noted. Neutrophils were present in lamina propria, dense and mixed type inflammatory cell infiltration was seen. These changes were limited thorough mucosa and submucosa. Granulomas and dysplasia were not encountered. The surgically resected specimen was compatible with UC (Figure 2). Acute phase proteins remained slightly increased and soiling was the major problem in the postoperative period. Anorectal manometry showed increased rectal sensitivity, significantly low resting anal sphincter pressure, normal rectoanal inhibitor reflex, and adequate pressure increase in voluntary sphincter contraction. However, perianal dermatitis and impaired perianal wound healing persisted (Figure 3).

Ileoscopy performed 8 mo after colectomy operation revealed a second lumen layer outside the lumen. Both lumen openings were hyperemic, edematous, and eroded (Figure 4). Ileal biopsies revealed backwash ileitis, without signs of Crohn's disease.

At the age of 18 years, the patient was referred

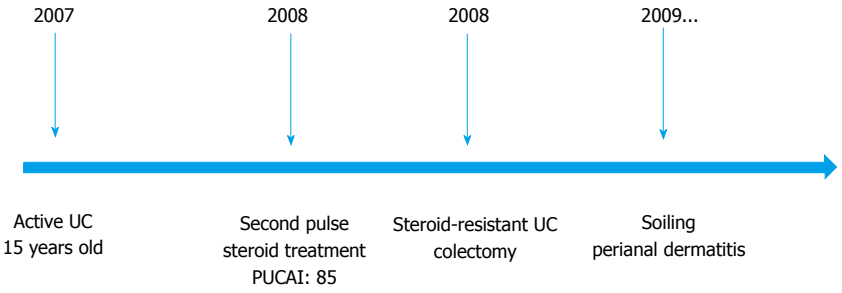


**Figure 1 The medical treatment and follow-up of the patient.** UC: Ulcerative colitis.

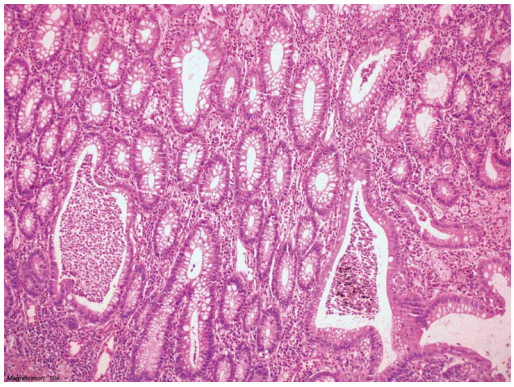

**Figure 2 Histopathologic appearance of colonic mucosa.**

**Figure 3 Perianal dermatitis and impaired perianal wound healing.**

to adult gastroenterology section. Pelvic magnetic resonance imaging and fistulography revealed an ileoileal fistula. At the age of 23 years, there was persistence of the perianal lesions and diarrhea despite treatment.

### *Genetic analysis*

To screen for monogenetic forms of IBD, we have performed whole exome sequencing of the patient sample using an exome enrichment kit (SureSelect V4UTR; Agilent) and next-generation sequencing device (Illumina HiSeq 2500). Analysis of rare genetic variants that affected protein sequence or interfered with mRNA transcription revealed a mutation in the *NCF2* gene (c.326A>G, p.Y109Y>C). Bioinformatic analysis of this sequence variant predicted that the amino acid exchange that was deleterious to the protein function.

A diagnosis of CGD was confirmed biologically by a nitroblue tetrazolium test (NBT) and flow cytometry-based dihydrorhodamine (DHR) or 2′7′-dichlorofluorescein diacetate assays. An NBT slide test with *Escherichia coli* lipopolysaccharide (840 W Sigma-Aldrich) was used to stimulate respiratory burst in phagocytic cells. Normally the percentage of blue-stained neutrophils in an NBT test (Figure 5) is close to 100 - but this percentage is close to 0 in CGD patients. In this patient, the NBT test confirmed a defective respiratory burst in the neutrophils. DHR test (Figure 6) uses flow cytometry to measure the oxidation of dihydrorhodamine 123 to rhodamine 123 in phorbol myrisate acetate-stimulated neutrophils, a marker for cellular NADPH oxidase activity. In this test the generation of hydrogen peroxide oxidizes

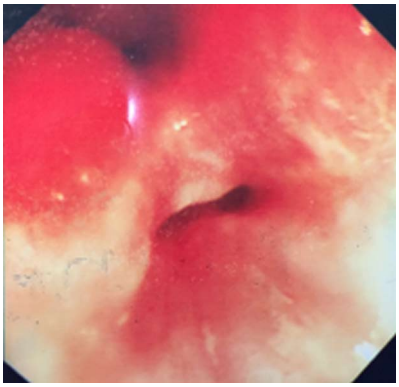

**Figure 4 Two lumens visualized during ileoscopy.**

the dye, leading to the emission of fluorescence. Mean fluorescence intensity of the activated cells correlates directly with (and thus serves as a reliable surrogate for) superoxide production. In this patient, the diagnosis of CGD was confirmed by using the NBT test and the DHR assay.

Based on the treatment-refractory course and the underlying primary immunodeficiency in the patient, we considered hematopoietic stem cell transplant (HSCT) for cure. The patient had been referred to the Department of Hematology and is currently being prepared for HSCT.

## DISSCUSSION

CGD is a primary immunodeficiency caused by a defect

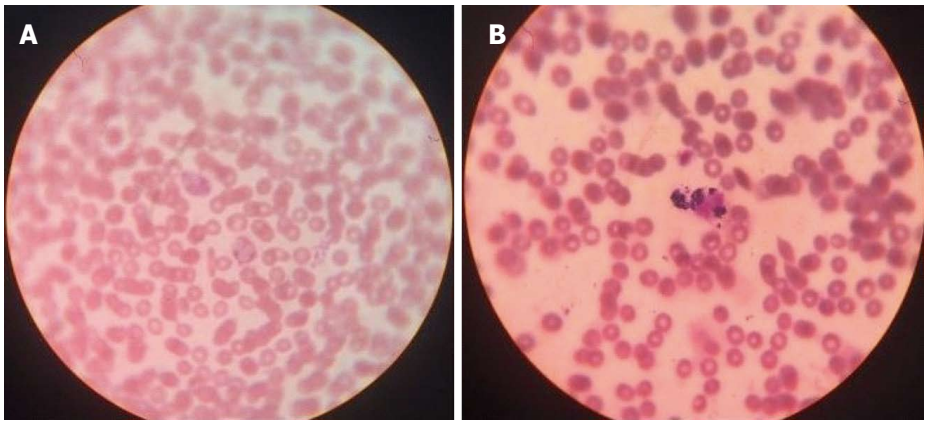


**Figure 5 Nitroblue tetrazolium test.** A: Neutrophils from a patient with CGD fail to reduce the NBT dye and appear clear; B: Normal (unaffected) cells reduce the NBT dye and stain blue/purple. CGD: Chronic granulomatous disease; NBT: Nitroblue tetrazolium test.

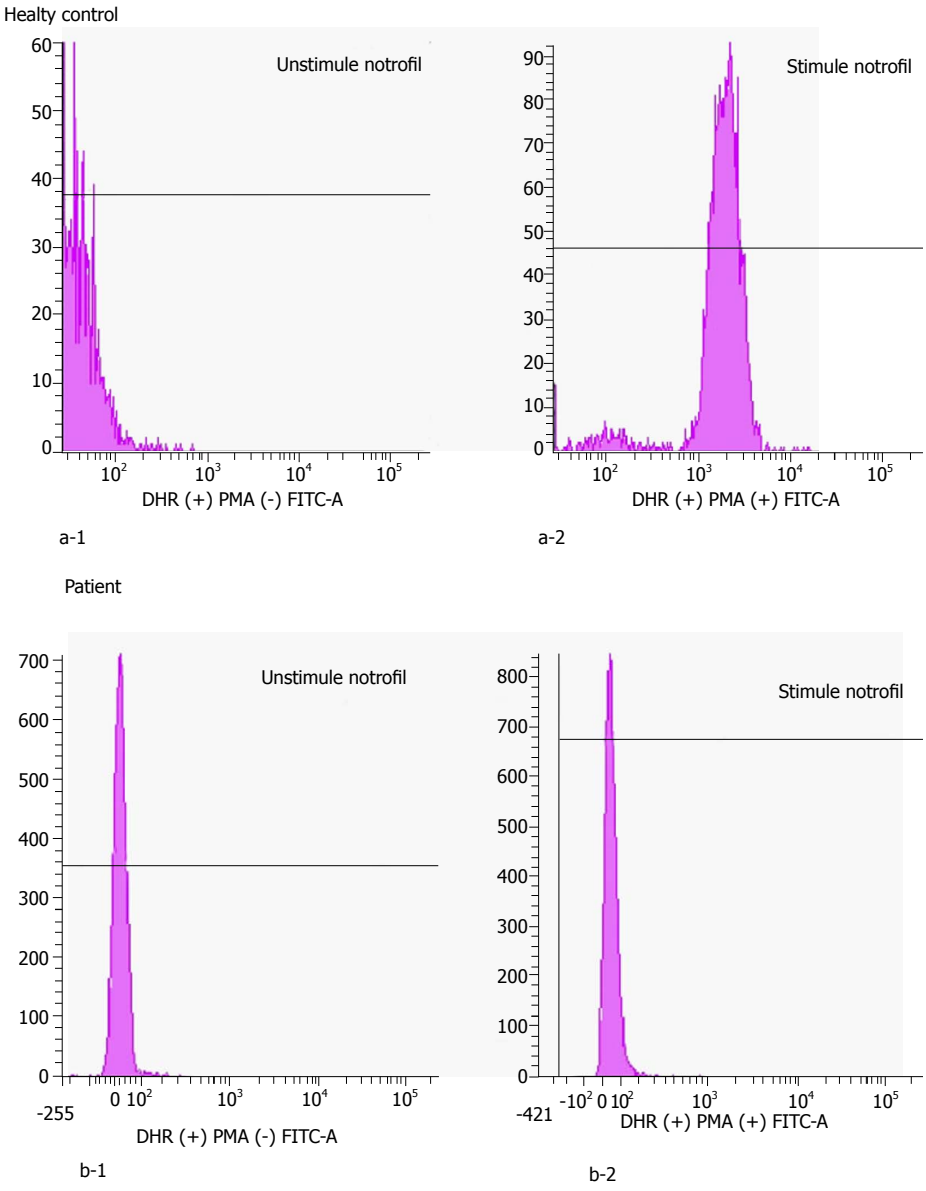


**Figure 6 Flow cytometry-based dihydrorhodamine test.** DHR: Dihydrorhodamine.

in a superoxide formation. More than 95% of cases are diagnosed before the age of 5 years. However, some patients may be diagnosed only in adulthood[1]. During evaluation of very early onset IBD under six years of

age, one can easily consider the presence of underlying immune deficiencies. However, it will be tough to consider this point in an adolescent patient with UC alone, normal physical development and negative history of recurrent infections like our patient.

Gastrointestinal tract involvement in CGD patients causes significant morbidity and mortality. Its phenotype might be similar in presentation-with IBD. Intestinal motility dysfunction, obstruction, ulceration and recurrent infection may occur along the entire tract[5]. Gastrointestinal involvement was reported in 32% to 48% of patients with CGD[1,6-8]. A study by van den Berg *et al*[6] found that 48% of CGD patients had experienced at least one episode of gastrointestinal manifestation. Colitis, perianal abscess and fistula formation may be the first symptoms for gastrointestinal involvement[9,10]. In our patient, the multiple colonoscopies revealed only large bowel involvement and normal terminal ileum. Therefore, the patient was monitored for years with a diagnosis of UC and eventually underwent colectomy due to the absence of response to medical therapy.

The endoscopic and pathological findings of patients with bowel involvement might be indistinguishable from that of IBD[10]. Cryptitis, crypt abscesses, eosinophilic infiltrates and granulomas may be detected in affected intestinal segments of CGD patients. Although presence of large pigment laden histiocytes in involved organs of CGD patients is significant, these findings are not specific or sensitive[11]. In our patient evaluation of colectomy specimens revealed no granulomas, therefore Crohn's disease or Crohn's-alike diseases were not considered. In addition, there were no recurrent bacterial and fungal infections or frequent antibiotic use. Immunologic tests for primary immunodeficiency were not considered due to the late onset of IBD. Eventually, unbiased whole exome sequencing revealed a homozygous missense mutation in NCF2, which was indicative of CGD diagnosis that was confirmed by NBT assay. Genetic studies showed NCF2 mutation for CGD which shows an autosomal recessive trend. Consanguineous marriage was not noted in family history, but coming from same village and the CGD disease with an autosomal recessive trait made us think of a distant relativity between parents.

Very early onset-IBD (VEO-IBD) (below-six years of age) has been shown to be associated with monogenetic etiologies in particular immune deficiencies[12]. Accordingly immunologic tests and genetic analysis are recommended in VEO-IBD patients. In IBD patients over the age of 6 years, initial search for immunologic parameters is recommended for the following reasons: (1) presence of lesions in the perianal region; (2) consanguineous parents; (3) unresponsive to treatment or steroid dependent; (4) family history of early-onset IBD; and (5) the presence of skin, nail, or hair abnormalities. With the rise in genetic studies, we learned that similar to IBD, immunodeficiencies like CGD may occur in after adolescence. Our experience on this case would make one wonder whether immunologic tests are necessary for every IBD patient, regardless of age.

In literature, basic immunologic tests as the first step in IBD patients were not recommended regardless of the presence of risk factors. However, considering the prolonged follow-up required of this chronic disease, we recommend otherwise.

## COMMENTS

### Case characteristics

Main symptoms were bloody mucoid diarrhea.

### Clinical diagnosis

Main clinical findings were perianal modifiers after colectomy operation.

### Differential diagnosis

Crohn's disease should be kept in mind in patients with perianal modifiers.

### Laboratory diagnosis

Elevated acute phase reactants and mutation in the *NCF2* gene (c.326A>G, p.Y109Y>C) were seen.

### Imaging diagnosis

Colonoscopic appearance was compatible with pancolitis.

### Pathological diagnosis

Histopathologic evaluation showed cryptitis, crypt abscesses, and pseudopolyps of the entire colonic mucosa; granulomas were not detected.

### Treatment

Treatment was consisted of steroids and immunosuppressive drugs.

### Experiences and lessons

Chronic granulomatous disease may present itself as ulcerative colitis in adulthood.

### Peer-review

This case report was well organized and well investigated. This paper will give us a new information especially in the field of inflammatory bowel disease.

## REFERENCES


1 **Winkelstein JA**, Marino MC, Johnston RB, Boyle J, Curnutte J, Gallin JI, Malech HL, Holland SM, Ochs H, Quie P, Buckley RH, Foster CB, Chanock SJ, Dickler H. Chronic granulomatous disease. Report on a national registry of 368 patients. *Medicine* (Baltimore) 2000; **79**: 155-169 [PMID: 10844935]

2 **Meischl C**, Roos D. The molecular basis of chronic granulomatous disease. *Springer Semin Immunopathol* 1998; **19**: 417-434 [PMID: 9618766]

3 **Segal BH**, Leto TL, Gallin JI, Malech HL, Holland SM. Genetic, biochemical, and clinical features of chronic granulomatous disease. *Medicine* (Baltimore) 2000; **79**: 170-200 [PMID: 10844936]

4 **Muise AM**, Xu W, Guo CH, Walters TD, Wolters VM, Fattouh R, Lam GY, Hu P, Murchie R, Sherlock M, Gana JC, Russell RK, Glogauer M, Duerr RH, Cho JH, Lees CW, Satsangi J, Wilson DC, Paterson AD, Griffiths AM, Silverberg MS, Brumell JH. NADPH oxidase complex and IBD candidate gene studies: identification of a rare variant in NCF2 that results in reduced binding to RAC2. *Gut* 2012; **61**: 1028-1035 [PMID: 21900546 DOI: 10.1136/gutjnl-2011-300078]

5 **Barton LL**, Moussa SL, Villar RG, Hulett RL. Gastrointestinal complications of chronic granulomatous disease: case report and literature review. *Clin Pediatr* (Phila) 1998; **37**: 231-236 [PMID: 9564572]

6 **van den Berg JM**, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L, Español T, Fischer A, Kurenko-Deptuch M, Mouy R, Petropoulou T, Roesler J, Seger R, Stasia MJ, Valerius NH, Weening RS, Wolach B, Roos D, Kuijpers TW. Chronic granulomatous disease: the European experience. *PLoS One* 2009; **4**: e5234 [PMID: 19381301 DOI: 10.1371/journal.pone.0005234]

7 **Holland SM**. Chronic granulomatous disease. *Clin Rev Allergy Immunol* 2010; **38**: 3-10 [PMID: 19504359 DOI: 10.1007/s12016-009-8136-z]

8 **Marciano BE**, Rosenzweig SD, Kleiner DE, Anderson VL, Darnell DN, Anaya-O'Brien S, Hilligoss DM, Malech HL, Gallin JI, Holland SM. Gastrointestinal involvement in chronic granulomatous disease. *Pediatrics* 2004; **114**: 462-468 [PMID: 15286231]

9 **Glocker E**, Grimbacher B. Inflammatory bowel disease: is it a primary immunodeficiency? *Cell Mol Life Sci* 2012; **69**: 41-48 [PMID: 21997382 DOI: 10.1007/s00018-011-0837-9]

10 **Alimchandani M**, Lai JP, Aung PP, Khangura S, Kamal N, Gallin JI, Holland SM, Malech HL, Heller T, Miettinen M, Quezado MM. Gastrointestinal histopathology in chronic granulomatous disease: a study of 87 patients. *Am J Surg Pathol* 2013; **37**: 1365-1372 [PMID: 23887163 DOI: 10.1097/PAS.0b013e318297427d]

11 **Barbato M**, Ragusa G, Civitelli F, Marcheggiano A, Di Nardo G, Iacobini M, Melengu T, Cucchiara S, Duse M. Chronic granulomatous disease mimicking early-onset Crohn's disease with cutaneous manifestations. *BMC Pediatr* 2014; **14**: 156 [PMID: 24947584 DOI: 10.1186/1471-2431-14-156]

12 **Uhlig HH**. Monogenic diseases associated with intestinal inflammation: implications for the understanding of inflammatory bowel disease. *Gut* 2013; **62**: 1795-1805 [PMID: 24203055 DOI: 10.1136/gutjnl-2012-303956]


**P- Reviewer**: Freeman HJ, Jamali R, Naito Y, Ruffolo C, Sipos F
**S- Editor**: Ji FF **L- Editor**: A **E- Editor**: Wu HL

World Journal of Gastrointestinal Pathophysiology

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

World J Gastrointest Pathophysiol 2017 May 15; 8(2): 93-95

DOI: 10.4291/wjgp.v8.i2.93

ISSN 2150-5330 (online)

CASE REPORT

# Duodenal localization of plasmablastic myeloma

Stefano Licci

Stefano Licci, Department of Pathology, Santo Spirito Hospital, 00193 Rome, Italy

Author contributions: Licci S made the histological diagnosis, reviewed the literature and conceived and wrote the case report.

Institutional review board statement: This case report was exempt from the institutional review board standards at Santo Spirito Hospital (Rome, Italy).

Informed consent statement: The patient involved in this study gave her written informed consent authorizing use and disclosure of her protected health information.

Conflict-of-interest statement: The author has no conflicts of interest to declare.



Manuscript source: Invited manuscript

Correspondence to: Stefano Licci, MD, Department of Pathology, Santo Spirito Hospital, Lungotevere in Sassia 1, 00193 Rome, Italy. stefano.licci@hotmail.it
Telephone: +39-6-68352585
Fax: +39-6-68352491

Received: November 4, 2016
Peer-review started: November 5, 2016
First decision: December 29, 2016
Revised: January 27, 2017
Accepted: March 12, 2017
Article in press: March 13, 2017
Published online: May 15, 2017

## Abstract

Gastrointestinal involvement in plasma cell neoplasms, either as primary localizations (extramedullary plasmacytomas) or as secondary involvement in systemic multiple myeloma, is a well-known event. Accurate histological examination is crucial in defining the diagnosis. In this report, an uncommon case of duodenal localization of myeloma with plasmablastic features is described, with emphasis on the role of clinical data and findings from ancillary immunostaining techniques to avoid misdiagnosis.

**Key words:** Gastrointestinal tract; Myeloma; Plasma cell neoplasm; Plasmablastic; Duodenum



**Core tip:** In cases of gastrointestinal involvement by high-grade plasma cell neoplasia, the presence of large atypical cells infiltrating the lamina propria of the mucosa may lead to an erroneous diagnosis of poorly differentiated carcinoma. Clinical data and findings from ancillary immunostaining techniques are crucial to avoid misdiagnosis.

Licci S. Duodenal localization of plasmablastic myeloma. *World J Gastrointest Pathophysiol* 2017; 8(2): 93-95 Available from: URL: http://www.wjgnet.com/2150-5330/full/v8/i2/93.htm DOI: http://dx.doi.org/10.4291/wjgp.v8.i2.93

## INTRODUCTION

Involvement of the gastrointestinal tract by plasma cell neoplasms is a well-known event, and many cases have been described in the literature, either as primary localizations (extramedullary plasmacytomas)[1] or as secondary involvement in systemic multiple myeloma[2]. In this report, the immunomorphological findings of an uncommon case of duodenal localization of myeloma with plasmablastic features are described.

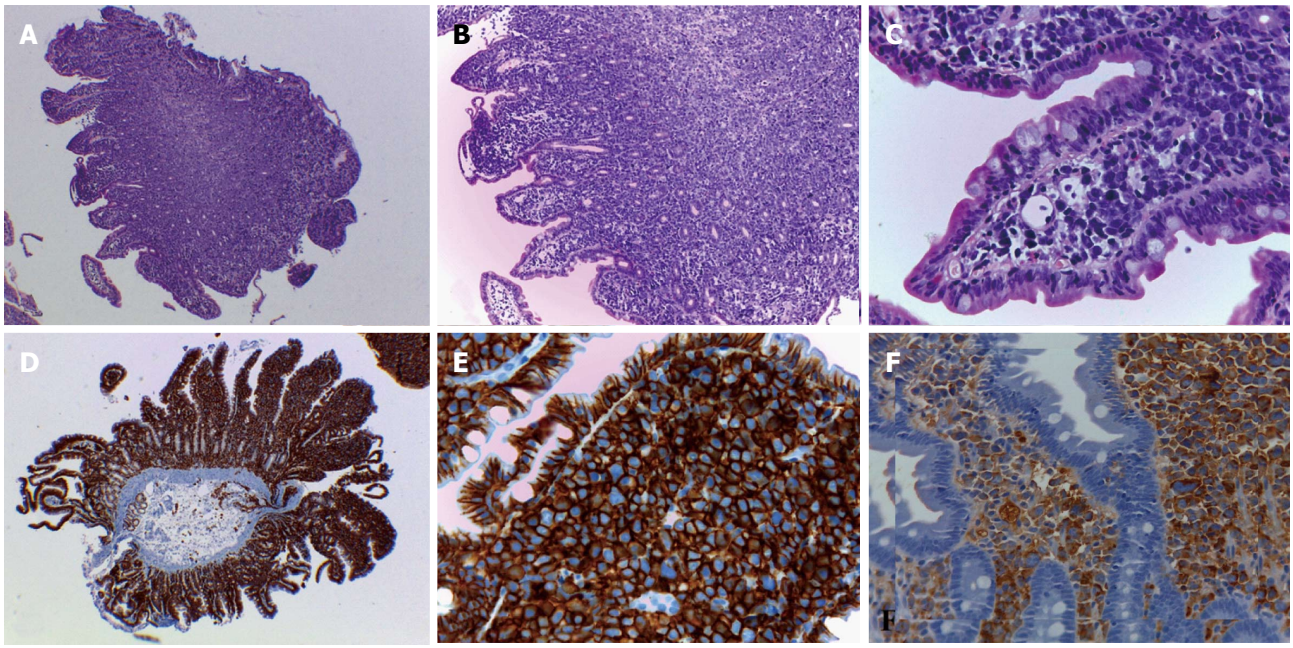


**Figure 1 Histological examination of duodenal mucosa biopsy samples.** A: Low-power histological examination of duodenal mucosa fragments shows a hypercellular inter-glandular stroma (hematoxylin-eosin, original magnification × 4); B, C: At medium- and high-power; B: Hematoxylin-eosin, original magnification × 10; C: Hematoxylin-eosin, original magnification × 40), an infiltration of the lamina propria by atypical cells is better appreciated, with neoplastic cells displaying medium to large size pleomorphic nuclei, sometimes with prominent nucleoli, and scanty cytoplasm; D-F: CD138 immunostaining shows a strong and diffuse membrane staining, revealing the plasma cell nature of the neoplastic infiltrate (D: Original magnification × 4; E: Original magnification × 40), with prevalent λ chain immunohistochemical expression (F: Original magnification × 40).

## CASE REPORT

A 60-year-old woman presented with unintentional weight loss, anemia and thrombocytopenia. Serum protein electrophoresis revealed a monoclonal peak of IgG (30 g/L), and immunofixation identified λ light chains. For better hematological evaluation, a bone marrow trephine biopsy was performed. Marrow spaces showed remarkably increased cellularity (about 90%), mainly represented by immature/atypical $CD138^+$ plasma cells with prevalent λ chain immunohistochemical expression (about 80% of the total cellularity). Accordingly, the diagnosis was plasma cell neoplasm, with morphological features consistent with "high-grade" plasma cell myeloma. The residual cellularity was composed of trilineage hematopoietic cells with reactive changes, rare $CD34^+$ blast cells and some B ($CD20^+$) and T ($CD3^+$) reactive small lymphocytes, showing interstitial and micronodular distribution.

In the days following the initial presentation to clinic, the patient re-presented with an acute worsening of anemia and an episode of melena. Suspected gastrointestinal bleeding was investigated by esophagogastroduodenoscopy; the esophageal and gastric mucosa appeared normal, while the duodenal mucosa was characterized by presence of multiple micropolyps, which were biopsied for histology. Microscopic examination revealed a diffuse infiltration in the lamina propria by medium- to large-sized cells with high nuclear-cytoplasmic ratio, atypical pleomorphic nuclei and prominent nucleoli, consistent with malignant neoplasia (Figure 1A-C). Immunohistochemical study excluded infiltration by poorly differentiated carcinoma (*i.e.,* AE1/3 cytokeratin immunostaining was negative) and revealed a diffuse and strong positivity for the CD138 plasma cell marker (Figure 1D and E). Further immunostaining analyses showed a prevalent λ chain expression (Figure 1F).

Ultimately, the diagnosis of duodenal localization of plasma cell neoplasm showing plasmablastic myeloma features was made on the basis of the cell morphology findings and consistent with the previous bone marrow trephine biopsy diagnosis.

## DISCUSSION

In cases of gastrointestinal plasma cell neoplasia, histological examination can represent a diagnostic pitfall, especially when the clinical data are missing; the underlying plasma cell neoplasia can remain unknown and the tumor cells can appear immature and/or atypical. In fact, the presence of neoplastic atypical cells diffusely infiltrating the lamina propria among glandular structures may be easily misdiagnosed as a poorly differentiated carcinoma. Furthermore, even in well differentiated cases with recognizable plasma cells, the issue can be complicated by the presence of numerous Russel bodies - spherical intracytoplasmic eosinophilic immunoglobulin - containing structures, easily detectable by microscopic observation-either in association with neoplastic plasma cell proliferation, in cases of lymphoproliferative disorders displaying a certain degree of plasma cell differentiation, or in non-neoplastic,

inflammatory processes characterized by a conspicuous plasma cell infiltrate, such as the so-called Russell body gastritis[3]. In such cases, plasma cells can take on the form of the signet-ring cells of poorly differentiated mucinous carcinoma of the gastrointestinal tract. Thus, careful microscopic observation must be integrated with ancillary techniques, mainly immunohistochemical staining analyses, to formulate the right diagnosis. Immunoreaction for cytokeratins is determinant for excluding a carcinoma, and demonstration of plasma cell [CD138$^+$ and/or CD38$^+$ and/or VS38c (plasma cell p63)$^+$] proliferation with κ or λ light-chain restriction enables distinction between a reactive and a neoplastic plasma cell infiltrate.

For the case presented herein, the clinical data represented an important tool for making the right diagnosis, similar to a previously described case of extramedullary plasmablastic myeloma of the small bowel[4]. The patient's experience of acute worsening of anemia and an episode of melena suggested gastrointestinal bleeding; esophagogastroduodenoscopy disclosed the duodenal mucosa lesions and the histological diagnosis were supported by the clinical-anamnestic data of a previous diagnosis of multiple myeloma. In such cases of secondary gastroenteric involvement by myeloma, therapy options include induction chemotherapy with immunomodulatory agents or proteasome inhibitors and corticosteroids. Stem cell transplantation may improve the remission rates and overall survival.

## COMMENTS

### Case characteristics

A 60-year-old woman with recent clinical history of multiple myeloma presented for acute worsening of anemia and an episode of melena.

### Clinical diagnosis

To address suspected gastrointestinal bleeding, an esophagogastroduodenoscopy was performed and revealed multiple micropolyps of duodenal mucosa, which were biopsied for histology.

### Differential diagnosis

Adenomatous duodenal polyps; Primary duodenal adenocarcinoma; Secondary duodenal involvement by myeloma; Other neoplasia.

### Laboratory diagnosis

Serum protein electrophoresis monoclonal peak of IgG for the underlying disease; Severe worsening of anemia.

### Imaging diagnosis

Bone osteolytic lesions for the underlying disease.

### Pathological diagnosis

Secondary duodenal localization of plasmablastic myeloma.

### Treatment

Proteasome inhibitors and corticosteroids; Stem cell transplantation.

### Related reports

Because of the possible morphological overlap, a secondary gastrointestinal localization of high-grade myeloma can be mistakenly diagnosed as poorly differentiated adenocarcinoma.

### Term explanation

The term "plasmablastic" in myeloma indicates a low degree of tumor cell differentiation, related to a more aggressive biological behavior.

### Experiences and lessons

Correlation of the histopathological findings with clinical and medical history is the basis of a correct diagnosis for plasmablastic myeloma with duodenal localization.

### Peer-review

The strength of this report lies in the importance of the histopathological study, deeply influenced by the knowledge of the clinical and medical history.

## REFERENCES


1 **Lopes da Silva R**. Extramedullary plasmacytoma of the small intestine: clinical features, diagnosis and treatment. *J Dig Dis* 2012; **13**: 10-18 [PMID: 22188911 DOI: 10.1111/j.1751-2980.2011.00544.x]

2 **Talamo G**, Cavallo F, Zangari M, Barlogie B, Lee CK, Pineda-Roman M, Kiwan E, Krishna S, Tricot G. Clinical and biological features of multiple myeloma involving the gastrointestinal system. *Haematologica* 2006; **91**: 964-967 [PMID: 16818286]

3 **Licci S**, Sette P, Del Nonno F, Ciarletti S, Antinori A, Morelli L. Russell body gastritis associated with Helicobacter pylori infection in an HIV-positive patient: case report and review of the literature. *Z Gastroenterol* 2009; **47**: 357-360 [PMID: 19358062 DOI: 10.1055/s-2008-1027656]

4 **Reddy R**, Vohra S, Macurak R. Secondary Extramedullary Plasmablastic Myeloma of the Small Bowel. *Clin Gastroenterol Hepatol* 2015; **13**: A23-A24 [PMID: 26122762 DOI: 10.1016/j.cgh.2015.06.029]


**P- Reviewer**: Mica L **S- Editor**: Qi Y **L- Editor**: A
**E- Editor**: Wu HL

World Journal of Gastrointestinal Pathophysiology

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

World J Gastrointest Pathophysiol 2017 May 15; 8(2): 96-99

DOI: 10.4291/wjgp.v8.i2.96

ISSN 2150-5330 (online)

CASE REPORT

# Late onset pulmonary metastasis more than 10 years after primary sigmoid carcinoma

Anne M Daniels, Jeroen F J Vogelaar

Anne M Daniels, Jeroen F J Vogelaar, Department of Surgery, VieCuri Medical Centre, 5912 BL Venlo, The Netherlands

Author contributions: Daniels AM was responsible for sample collection, acquisition of patient data, drafting and revising of the manuscript; Vogelaar JFJ planned the study and revised the manuscript.

Institutional review board statement: Approval from the Local Ethical Committee for this study was granted.

Informed consent statement: Informed consent was obtained from the patient.

Conflict-of-interest statement: Both authors have no conflicts of interests to declare.



Manuscript source: Unsolicited manuscript

Correspondence to: Anne M Daniels, BSc, Department of Surgery, VieCuri Medical Centre, Tegelseweg 210, 5912 BL Venlo, The Netherlands. annedaniels1991@gmail.com
Telephone: +31-77-320555
Fax: +31-77-325309

Received: October 25, 2016
Peer-review started: October 27, 2016
First decision: December 1, 2016
Revised: January 3, 2017
Accepted: March 12, 2017
Article in press: March 13, 2017
Published online: May 15, 2017

## Abstract

According to current guidelines, follow-up of patients with colorectal cancer is ended after five years. Also, chest X-ray is not part of standard investigation during follow-up. We describe a case of a 74-year-old patient, more than ten years after a sigmoid resection because of carcinoma of the sigmoid. No recurrence was detected during intensive follow-up. However, ten years after resection of the sigmoid adenocarcinoma, complaints of coughing induced further examination with as result the detection of a solitary metastasis in the left lung of the patient. Within half-a-year after metastasectomy of the lung metastasis, she presented herself with thoracic pain and dyspnea resulting in discovering diffuse metastasis on pulmonary, pleural, costal and muscular level. Five year follow-up of colorectal carcinoma without chest X-ray can be questioned to be efficient. The growing knowledge of tumor biology might in future adjust the duration and frequency of diagnostic follow-up to prevent (late) recurrence in patients with colorectal carcinoma.

**Key words:** Colorectal cancer; Metastasis; Tumor biology; Tumor dormancy; Follow-up



**Core tip:** This case report presents a case of a woman with pulmonary metastases 10 years after a sigmoid resection. No recurrence of the primary intestinal tumor was detected during follow-up. Upcoming knowledge on tumor behavior based on its biology might give new insights in late-onset metastases. Therefore, follow-up protocols and current therapy guidelines might have to be re-evaluated.

Daniels AM, Vogelaar JFJ. Late onset pulmonary metastasis more than 10 years after primary sigmoid carcinoma. *World J Gastrointest Pathophysiol* 2017; 8(2): 96-99 Available from: URL: http://www.wjgnet.com/2150-5330/full/v8/i2/96.htm DOI: http://dx.doi.org/10.4291/wjgp.v8.i2.96

## INTRODUCTION

Colorectal carcinoma (CRC) is the most common type of gastrointestinal cancer. The current screening methods enable early detection, for example adenomatous polyps, to prevent the development of invasive carcinoma[1]. Etiology of CRC comprises genetic involvement, molecular events, environmental factors and inflammatory conditions[2]. CRC usually develops over a period of 10-15 years[3], most often affecting people aged over 50[4]. After a curative resection, prognosis of CRC is worse in poorly differentiated, mucinous carcinomas with large invasion depth and in the presence of lymph node metastases[5]. Follow-up of patients with CRC according to the national guidelines consists of echography or computed tomography (CT) of the abdomen, colonoscopy and determination of carcinoembryonic antigen (CEA) until five years after surgical resection[6].

CRC can spread by lymphatic and hematogenous dissemination, as well as by contiguous and transperitoneal routes. Metastasis, at initially diagnosis present in 20% of all patients, is the main cause of mortality in patients with CRC[7,8]. When cancer spreads to one single organ, the malignancy presents itself with a specific biological profile and clinical characteristics. The site of the metastatic disease influences the prognosis and the possibilities for treatment. Organs most often affected by CRC are the liver and the lungs[9]. For pulmonary lesions, the criteria for resectability of the metastatic lesion comprise; control of the primary tumor, possible complete resection and adequate pulmonary reserve to tolerate the planned resection[10].

## CASE REPORT

Ten years ago, a 63-year-old woman was admitted to our hospital with changes in bowel habits. At that time, she had no medical history of abdominal complaints or surgical operations. Her father was familiar with lung carcinoma, her mother and both of her sisters suffered from breast carcinoma.

A colonoscopy reveals a sigmoid colon stricture with unknown length due to the impossibility of endoscopically passing the stricture. Biopsy of the sigmoid shows a poorly differentiated adenocarcinoma. Computed tomography and echography of the liver, spleen and of the pelvic, cardio-thoracic, para-aortic and paracaval region show no lesions suspected for malignancy. An uncomplicated surgical resection of the sigmoid took place. The tumor, with a maximum diameter of five centimeters, including five surrounding lymph nodes and infiltrated fat tissue were removed in totality. No lymph node metastases were detected.

One year after the surgical resection, biopsy of a polyp of the ascending colon showed a tubular adenoma with low grade dysplasia. During further follow up of this patients no malignancies or recurrent lesions were disclosed. The CEA was tested every three months with a maximum value of 2.2 mcg/L. The intensive follow-up period was completed five years after sigmoid resection.

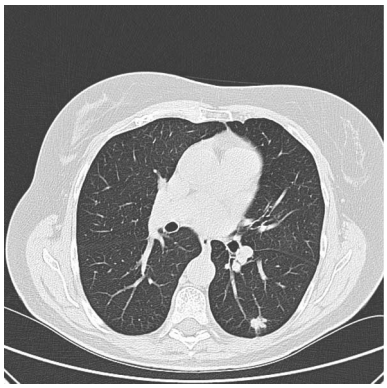

**Figure 1 Computed tomography of the pulmonary lesion.**

Ten years after resection of the adenocarcinoma located in the sigmoid without recurrent carcinoma's during follow up, our patient presented herself with persistent coughing. Imaging of the thorax shows a lesion of 1.5 centimeters located in the inferior lobe of the left lung (Figure 1). Biopsy reveals an adenocarcinoma with positive CDX-2 staining, corresponding with intestinal origin of the cells. The pulmonary tumor was removed by video assisted thoracic surgery (VATS), presumably in totality. Colonoscopy shows no metachronous neoplasia. Within half a year, she was admitted to our emergency department with complaints of pain on the left side of her chest resulting in dyspnea, the pain was coherent with breathing movement and pain radiation towards her spine. CT-thorax shows extensive pleural, costal and muscular metastases (Figure 2). To reduce pain, palliative chemotherapy and additional radiotherapy were started.

## DISCUSSION

Early detection of relapse or metastatic disease can be effectuated by intensive follow up during five years as recorded in the directive of Oncoline[6]. This follow-up comprises echography and optional CT-abdomen twice a year during 1-2 years, followed by once a year up to 5 years after the surgical resection. CEA should be determined every 3-6 mo during 3 years, followed by every 6 mo until 5 years after treatment. Colonoscopy is performed three, six and twelve months after surgical resection. Based on the number, localization and size of the lesions, repetition of colonoscopy is indicated after 3 or 5 years[6].

However, several clinical and experimental observations suggest that metastases can develop even in the absence of a detectable primary tumor. Both large and small tumors have the capability to metastasize[11,12]. Dissemination of tumor cells can occur in pre-invasive stages of tumor progression resulting in the development of early dormant disseminated tumor cells (DTCs). Early DTCs can remain dormant for a long period with

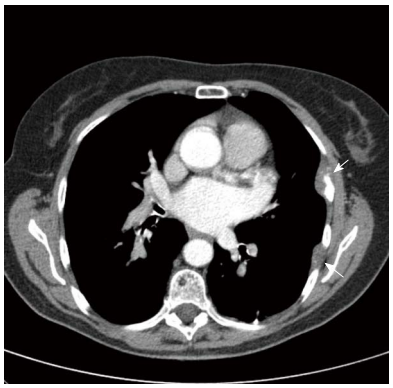

**Figure 2 Computed tomography of the pleural metastasis (indicated with arrows).**

manifestation of metastatic growth as result[13,14]. Metastases may thus be initiated by and evolve from dormant DTCs, rather than established primary tumors. These DTCs can generate metastases with different characteristics from those of the primary tumor. The discriminating characteristics may explain the lack of success treating metastases with therapies based on primary tumor characteristics[14]. Clinical evidence supports that the vast majority of early DTCs seem to be dormant[13].

In conclusion, five year follow-up of colorectal carcinoma without chest X-ray can be questioned to be efficient with the upcoming knowledge of early DTC's. Adjustment of the duration and frequency of diagnostic follow-up and adjustment of treatment might be effective for early detection of metastatic disease and prevention of late recurrence in patients with colorectal carcinoma.

## ACKNOWLEDGMENTS

The authors wish to thank Simons P, MD, PhD for providing the CT-scan figures.

## COMMENTS

### Case characteristics

A 63-year-old woman presented with a poorly differentiated adenocarcinoma of the sigmoid as primary tumor. No recurrence of the primary intestinal tumor was detected during follow-up. Ten years after resection of the primary tumor, computed tomography reveals a pulmonary metastasis in the left lung of the patient.

### Clinical diagnosis

Persistent cough.

### Differential diagnosis

The most common causes of chronic cough are postnasal drip, asthma, and acid reflux from the stomach. Less common causes include infections, medications, and lung diseases (*e.g.*, primary/secondary malignancy).

### Laboratory diagnosis

No raised CRP or ESR. Normal leucocyte count. Carcinoembryonic antigen 1.7 mcg/L.

### Imaging diagnosis

Computed tomography (CT) shows a lesion of 1.5 centimeters located in the inferior lobe of the left lung.

### Pathological diagnosis

Pulmonary metastasis of intestinal origin; a poorly differentiated adenocarcinoma.

### Treatment

Metastasectomy by video assisted thoracic surgery.

### Related reports

Follow-up of colorectal carcinoma consists of echography, optional CT-abdomen, CEA determination and colonoscopy. Without recurrence of the tumor within five years after primary resection, intensive follow-up is ended. However, several clinical and experimental observations suggest that metastases can develop even in the absence of a detectable primary tumor, based on the principle of dormant disseminated tumor cells.

### Term explanation

Late onset pulmonary metastases more than ten years after primary colorectal cancer, without recurrence of the primary tumor, is very rare.

### Experiences and lessons

Most recent guidelines recommend follow-up until five years after treatment of colorectal cancer. A pulmonary mass on imaging of the thorax, even more than 10 years after curative resection, is suspicious for metastatic disease. In general, more insights in the behavior of cancer (tumor biology) might change follow-up regimens in future.

### Peer-review

The authors presented a case of a woman with pulmonary metastases 10 years after a sigmoid resection. This is clinically important and the paper is well written.

## REFERENCES

1 **Desch CE**, Benson AB, Somerfield MR, Flynn PJ, Krause C, Loprinzi CL, Minsky BD, Pfister DG, Virgo KS, Petrelli NJ. Colorectal cancer surveillance: 2005 update of an American Society of Clinical Oncology practice guideline. *J Clin Oncol* 2005; **23**: 8512-8519 [PMID: 16260687 DOI: 10.1200/JCO.2005.04.0063]

2 **Dragovich T**, Tsikitis VL, Talavera F, Espat NJ, Schulman P. Colon Cancer. Medscape 2016 Available from: URL: http://emedicine.medscape.com/article/277496-overview

3 **Kelloff GJ**, Schilsky RL, Alberts DS, Day RW, Guyton KZ, Pearce HL, Peck JC, Phillips R, Sigman CC. Colorectal adenomas: a prototype for the use of surrogate end points in the development of cancer prevention drugs. *Clin Cancer Res* 2004; **10**: 3908-3918 [PMID: 15173100 DOI: 10.1158/1078-0432.CCR-03-0789]

4 **Howlader N**, Noone AM, Krapcho M, Garshell J, Miller D, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z,Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2012, National Cancer Institute. Bethesda, MD, United States. Available from: URL: http://seer.cancer.gov/csr/1975_2012/

5 **Kumar V**, Abbas AK, Fausto N, Aster JC. Robbins and Cotran Pathologic Basis of Disease, Professional Edition: Elsevier Health Sciences; 2014

6 **Comprehensive Cancer Centre The Netherlands**. Dutch guideline "colorectal carcinoma". 2016

7 **Van Cutsem E**, Oliveira J. Advanced colorectal cancer: ESMO clinical recommendations for diagnosis, treatment and follow-up. *Ann Oncol* 2009; **20** Suppl 4: 61-63 [PMID: 19454465 DOI: 10.1093/annonc/mdp130]

8 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer*

*J Clin* 2016; **66**: 7-30 [PMID: 26742998]
9 **Vatandoust S**, Price TJ, Karapetis CS. Colorectal cancer: Metastases to a single organ. *World J Gastroenterol* 2015; **21**: 11767-11776 [PMID: 26557001 DOI: 10.3748/WJG.v21.i41.11767]
10 **Warwick R**, Page R. Resection of pulmonary metastases from colorectal carcinoma. *Eur J Surg Oncol* 2007; **33** Suppl 2: S59-S63 [PMID: 18023132 DOI: 10.1016/j.ejso.2007.09.018]
11 **van de Wouw AJ**, Janssen-Heijnen ML, Coebergh JW, Hillen HF. Epidemiology of unknown primary tumours; incidence and population-based survival of 1285 patients in Southeast Netherlands, 1984-1992. *Eur J Cancer* 2002; **38**: 409-413 [PMID: 11818207 DOI: 10.1016/S0959-8049(01)00378-1]
12 **Engel J**, Eckel R, Kerr J, Schmidt M, Fürstenberger G, Richter R, Sauer H, Senn HJ, Hölzel D. The process of metastasisation for breast cancer. *Eur J Cancer* 2003; **39**: 1794-1806 [PMID: 12888376 DOI: 10.1016/S0959-8049(03)00422-2]
13 **Hüsemann Y**, Geigl JB, Schubert F, Musiani P, Meyer M, Burghart E, Forni G, Eils R, Fehm T, Riethmüller G, Klein CA. Systemic spread is an early step in breast cancer. *Cancer Cell* 2008; **13**: 58-68 [PMID: 18167340 DOI: 10.1016/j.ccr.2007.12.003]
14 **Sosa MS**, Bragado P, Aguirre-Ghiso JA. Mechanisms of disseminated cancer cell dormancy: an awakening field. *Nat Rev Cancer* 2014; **14**: 611-622 [PMID: 25118602 DOI: 10.1038/nrc3793]

**P- Reviewer**: Akiho H, Deng CX, Peinado MA **S- Editor**: Gong ZM
**L- Editor**: A **E- Editor**: Wu HL

Published by Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com