GAVIN PUBLISHERS

Journal of Oncology Research and Therapy

Ponzetto A, et al. J Oncol Res Ther: JONT-128.

Research Article

DOI: 10.29011/2574-710X.000028

Pathogenic Strains of *Helicobacter pylori* in Patients with Hepatocellular Carcinoma

Antonio Ponzetto[1*], Tito Livraghi[2], Maria Franca Meloni[3], Elena Moretti[4], Tommaso Stroffolini[5], Natale Figura[5]

[1]Department of Medical Sciences, University of Torino, Italy

[2]Department of Radiology, Istituto Clinico Humanitas, Rozzano, Italy

[3]Department of Radiology, Ospedale Valduce, Como, Italy

[4]Department of Molecular and Developmental Medicine, University of Siena, Italy

[5]Department of Medical, Surgical and Neurological Sciences, University of Siena, Italy

[*]**Corresponding author:** Antonio Ponzetto, Department of Medical Sciences, University of Torino, Corso AM Dogliotti 14, 10126 Torino, Italy. Tel: +390116708483; Fax: +390116334515; Email: antonio.ponzetto@unito.it

Citation: Ponzetto A, Livraghi T, Meloni MF, Moretti E, Stroffolini T, et al. (2017) Pathogenic Strains of Helicobacter pylori in Patients with Hepatocellular Carcinoma. J Oncol Res Ther: JONT-128. DOI: 10.29011/2574-710X.000028

Received Date: 14 July, 2017; **Accepted Date:** 19 July, 2017; **Published Date:** 26 July, 2017

Abstract

Background: Chronic hepatitis in humans can lead to Hepatocellular Carcinoma (HCC) after persistent cirrhosis. *Helicobacter pylori* (*H. pylori*) infection has been reported to occur very frequently in patients with cirrhosis. *Helicobacter* spp. have been correlated with acute and chronic hepatitis in Rhesus monkeys, cats and dogs, and experimental infection by *Helicobacter hepaticus* caused Hepatocellular Carcinoma (HCC) in male A/JCr mice following chronic hepatitis. Therefore, we performed a study to assess the seroprevalence of pathogenic *H. pylori* infection in Italian patients with HCC that developed following liver cirrhosis**.**

Methods: A total of 220 patients (160 males, 60 females, age range 33-88, year old) with HCC and 409 control patients (213 males, 196 females, age range 30-79-year-old), who presented consecutively to the Emergency Department of our Hospital, were examined. A serum sample from each patient was tested, by ELISA, for the presence of IgG antibodies to *H. pylori* and, by immunoblotting, for antibodies against The Cytotoxin-Associated Gene A (CagA) protein.

Results: Seropositivity to *H. pylori* was found in 183 out of 220 patients with HCC (83.1%) and in 220 of 409 controls (53.7%) ($p < 0.0001$) (OR 3.97; 95% CI 2.60-6.06). Anti-CagA seropositivity was found in 75.4% of HCC patients (89/118) and in 9.20% of controls (38/409) (OR 29.96; 95% confidence interval CI: 16.98-53.23); 87.5% of females and 70.9% of males with HCC had circulating anti-CagA antibodies, compared to 16.3% and 2.80% of control patients, respectively.

Conclusion: *H. pylori* infection and antibodies to CagA were found much more frequently in patients with HCC than in controls. Pathogenic strains of *H. pylori* could represent a notable co-factor for maintaining inflammation, thereby increasing the rate of cancer progression in cirrhotic patients.

Introduction

Hepatocellular Carcinoma (HCC) is the third cause of cancer death worldwide [1], and its prevalence has been steadily rising in the USA [2] and in the UK [3], but has now reached a plateau in the USA [4]. HCC is a frequent complication of chronic liver inflammation (Hepatitis) and of its long-term sequela, namely liver cirrhosis [5]. Chronic inflammation results from a variety of pathogenic insults, including metabolic disorders, autoimmunity, toxic or infectious diseases [5]. In Italy, it has been reported that 72.1% of all cirrhosis patients have antibodies to Hepatitis C Virus (HCV) in the serum, and up to 13% carry Hepatitis B Virus (HBV) [6]. Long-term follow-up studies of HBV and HCV infected individuals showed that a proportion of carriers (from 0% to 25%) develop cirrhosis and ultimately HCC [6-11]. However, it was shown that, in individuals of the general population in Benevento, aged 60 or above, 42% were infected with HCV; in Catanzaro, 34% of the general population of the same age were HCV-infected, and 50% of individuals in a Sicilian town were infected with HCV, but cirrhosis was infrequent, with only a small minority developing HCC [12-14]. In strong contrast, in Trieste, the occurrence of cirrhosis and HCC was very high despite a very low prevalence of HCV infection [15].

Host characteristics, in particular HLA haplotype, have been shown to influence the outcome of viral infection and the development of cirrhosis; this observation was first made by us and then unanimously confirmed [16-20]. Nevertheless, monozygotic twins have been reported to have discordant manifestations of hepatitis C, thus suggesting that factors that play a role in determining the outcome of HCV infections still remain to be clarified [21].

Viral infection, by itself, can only partially explain the pathogenesis of HCC, and HCV, in particular, appears to lack a transforming mechanism. Conversely, a variety of causes leading to chronic inflammation of the liver have been reported to lead to HCC, including iron overload [22,23], metabolic liver diseases [24,25], and food-derived toxins [26,27], even in the absence of virus carriage.

A new cause of liver inflammation (hepatitis) was discovered at the National Cancer Institute (NCI) during studies of chemical carcinogenesis; it consists of a Gram-negative bacterium, living in the bile canaliculi, thereafter named *Helicobacter hepaticus* (*H. hepaticus*) [28]. Experimental feeding of laboratory mice with *H. hepaticus* induced strong inflammatory changes (hepatitis) in the liver parenchyma, leading (rapidly) to HCC [28]. Several other *Helicobacter* species have been identified, that live in the bile canaliculi, not only of mice but also of chickens, Rhesus monkeys and other mammals, namely *H. pullorum, H. cinaedi, H. bilis,* and *H cholecystus* [29-32]. Of these bacteria, some have been detected in the bile of Chilean women suffering from chronic cholecystitis, thus showing that these pathogens are not restricted the host in which they were originally discovered [33]. Indeed, biliary tract and gallbladder cancer have been shown to be correlated with infection by *Helicobacter* spp [34-36].

A bacterium belonging to the same genus, *Helicobacter pylori* (*H. pylori*), was defined by the International Agency for Research on Cancer as a class I carcinogen. Indeed, infection by this pathogen leads to gastric cancer and to MALT lymphoma in humans [37].

H. pylori affects intracellular signal conduction in host cells, leading to activation of transcriptional factors via activation of the NF-Kappa B (NFkB) pathway [38,39]. The cag Pathogenicity Island (PAI) genes of *H. pylori* and their products are responsible for activating Mitogen Activated Protein-Kinases (MAP-kinases) [40], which in turn are strong activators of the nuclear proto-oncogenes c-FOS and c-JUN [41]; these contribute to the enhanced cell proliferation induced by *H. pylori*. Both NF-kB and MAP-kinase cascades are known to upregulate cyclin D1 expression, and overexpression of cyclin D1 shortens G1 phase and increases the rate of cell proliferation [42]. *H. pylori* in fact activates the cyclin D1 gene in a time- and dose-related manner, thus accounting for *H. pylori*-induced cell proliferation [43].

NF-kB activation is known to induce strong up-regulation of inflammation [44,45], and indeed *H. pylori* infection induced NF-kB activation correlates with an intense pro-inflammatory status [46], characterized by elevated levels of interleukin-1, interleukin-6, and interleukin-8 (IL-1, IL-6, IL-8) and of Tumor Necrosis Factor alpha (TNF-α). El-Omar, et al. [47] reported that genetic polymorphism for Interleukin-1 Beta (IL-1b) receptor is a key factor for developing gastric cancer following infection by *H. pylori.* However, recent meta-analyses and reviews have shown that pathogenic strains of the bacterium are the most important factor linked to cancer development as recently reported [48]. Since pathogenic strains of the bacterium increase inflammation, this strikingly emphasizes that pronounced inflammation in response to bacterial infection may determine which individuals succumb to gastric cancer after *H. pylori* infection.

Chronic inflammation of the liver parenchyma is a key feature of hepatitis, and can be derived from toxic, metabolic, autoimmune or infectious causes; henceforth we hypothesized that any chronic infection leading to NF-kB pathway activation (i.e. the pro-inflammatory response) would ultimately result in an increased risk of liver cirrhosis and progression to cancer. We previously analyzed the liver tissue obtained from patients with HCC at surgery for sequences of *Helicobacter* spp., and found them to be almost invariable [49]; sequences from dead bacteria could enter the liver via venous blood from the gut organs. Nevertheless, our findings were confirmed [50], leading us to believe that *H pylori* infection could play a role in the establishment or progression of HCC in humans. *H pylori* infection was reported as very common

in patients with liver cirrhosis [51-54], the precursor mechanism leading to HCC.

Therefore, we decided to verify the hypothesis that antibodies to *H pylori* would be highly prevalent in patients affected by HCC. We report that human patients with established HCC have a significantly higher prevalence of antibodies to *H. pylori* than controls. The relevance of this study was increased by the consideration that the persistence of antibodies to *H. pylori* often corresponds to chronic *H. pylori* infection.

Patients and Methods

The presence of anti-*H. pylori* antibodies was evaluated in 220 consecutive patients (160 males, 60 females, mean age 67 years old, range 33-88 years old) treated for HCC, developed following liver cirrhosis**,** at the Department of Radiology, Ospedale Civile di Vimercate, Italy, (MFM, TL).

HCC was either diagnosed by serum alpha fetoprotein detection, ultrasonography, spiral Computed Tomography (CT), or histology. Angiography, Magnetic Resonance Imaging (MRI) and Lipiodol-CT scans were performed when it was considered necessary to plan for a surgical therapy. A total of 43 out of the 220 cirrhotic patients were infected by HBV, 19 were infected by both HBV and HCV and 158 by HCV only. HBV infection was diagnosed by positivity of HBsAg and HBV-DNA in serum samples. Levels of HBsAg, anti-HBsAg and immunoglobulin M anticore (IgM anti-HBc), HBeAg, anti-HBe were determined by commercial kits (Axsym® System, Abbott Diagnostics, Maidenhead, UK). For detection and quantitative measurements of HBV-DNA in serum by the polymerase chain reaction and DNA hybridization, we used the Amplicor HBV Monitor™ Test (Roche Diagnostics System, Inc., Branchburg, USA).

HCV infection was diagnosed by positivity in serum samples for anti HCV (EIA, Ortho HCV 3.0, Ortho clinical Diagnostics, Neckarcemünd, Germany), confirmed by RIBA II (Ortho HCV RIBA II, Ortho clinical Diagnostics, Neckarcemünd, Germany) and by circulating HCV-RNA detected by the polymerase chain reaction (PCR, Amplicor Roche, Roche Diagnostics System, Inc., Branchburg, USA). The controls were 409 sexes- and age-matched patients consecutively admitted to the Department of Emergency Care of San Giovanni Battista Hospital of Torino (213 males, 196 females, age range 30-79 years old). Any patients having a diagnosis at discharge of bleeding duodenal ulcer or acute myocardial infarction were not considered, as these diseases have been shown to be linked to *H. pylori* infection in our series [55-57]. A commercial enzyme immunosorbent assay (ELISA, Helori-test® Eurospital, Trieste Italy), with a sensitivity of 94% and a specificity of 87%, was used to detect serum anti-*H. pylori* (IgG) antibodies [58].

Antibodies against the protein product of the Cytotoxin-associated gene A (anti-CagA) were measured by immunoblotting using a commercial assay (Nurex srl, Sassari, Italia).

Statistical Evaluation

The prevalence of *H. pylori* and anti-CagA antibodies in HCC patients and controls was compared using the chi-square test (χ^2) by means of a 2×2 contingency table. Results were considered significant when $p < 0.05$. In addition, Student's t test and regression straight lines correlation study were also employed where appropriate. The software EpiInfo version 5 Statcalc was used to calculate statistics.

Results

The presence of CagA antibodies in serum samples was detected in 89 of 118 (75.4%) HCC patients and in 38 of 409 (9.29%) control patients admitted to the emergency room ($p < 0.0001$; Odds Ratio OR 29.96; 95% confidence interval CI 16.98-53.23). Anti-CagA was found in 87.5% (28/32) of females treated for HCC, compared to 16.3% (32/196) of controls (OR 35.88; 95% CI 10.93-130.30); 61/86 (70.9%) males with HCC had antibodies against CagA compared to 2.80% of controls (6/213) (OR 30.91; 95% CL 30.91-242.50).

H. pylori infection was detected in 83.2% (183/220) of HCC patients, and 55.50% (227/409) in the Italian patients consecutively admitted to the emergency room ($p < 0.0001$) (OR 3.97, 95% confidence interval 2.60-6.06) (Table 1) and who complied with inclusion criteria (i.e. absence of bleeding duodenal ulcer or acute myocardial infarction). Of the female HCC patients, 51/60 (85.0%) were seropositive versus 105 of 196 (53.57 %) of female controls ($p < 0.0001$) (OR 4.91, 95% CI 2.18-11.37); 132/160 (82.5%) of male patients were seropositive versus 122 of 213 (57.27%) controls ($p < 0.0001$) (OR 3.52, 95% CI 2.10-5.92).

HCC Hp(+)/tot	Controls Hp(+)/tot	p	OR	CL
Males 132/160 (82.5%)	122/213 (57.27%)	< 0.0001	3.52	2.10-5.92
Females 51/60 (85.0%)	105/196 (53.57%)	< 0.0001	4.91	2.18-11.37
Total 183/220 (83.18%)	227/409 (55.50%)	<0.0001	3.97	2.60-6.06

HCC: consecutively treated patients with hepatocellular carcinoma. **Controls:** consecutive Italian patients admitted to the emergency Room, excluding those with acute myocardial infarction or acute duodenal ulcer bleeding. p: statistical significance (Yates corrected test). **OR:** Odds Ratio. **CL:** 95% Confidence Limit.

Table 1: Seroprevalence of anti-*H. pylori* antibodies in patients with hepatocellular carcinoma (HCC) and controls.

In addition, we highlight that was no relationship between the prevalence of antibodies to *H. pylori* and Child's class of severity of cirrhosis, according to Pugh et al., [59] and the reported etiology of the liver disease.

Finally, evaluating the seroprevalence of *H. pylori* in patients and control subjects, divided by decade of age, i.e.: 30-39 years; 40-49; 50-59; 60+, we rarely observed cases of HCC prior to 50 years of age, as expected, and a significant difference in *H. pylori* seroprevalence (patients vs. controls) was only found in the groups of 50-59 years ($p < 0.05$) and of 60+ years ($p < 0.00001$). Moreover, in both groups, *H. pylori* seropositivity increased with age, though with a higher rate in the HCC patients, compared to the control subjects. In fact, a comparison between regression straight lines showed analogous slopes (slope comparison: $t = 0.744$; $p < 0.05$) Table 2.

HCC anti-CagA(+)/tot	Controls anti-CagA(+)/tot	P	OR	CL
male 61/ 86 (70.93 %)	6/213 (2.81%)	< 0.0001	84.18	30.91-242.50
female28/ 32 (87.5%)	32/196 (16.3%)	< 0.0001	35.88	10.93-130.30
Total 89/118 (75.4%)	38/409 (9.29%)	<0.0001	29.96	16.98-53.23

HCC: all consecutively treated patients with hepatocellular carcinoma. Testing for anti-CagA was performed on all sera from one single year.
Controls: all consecutive Italian patients admitted to emergency Room, but Acute myocardial infarction and acute duodenal ulcer bleeding patients
P: statistical significance (Yates corrected test).
CL: 95% confidence limit.

Table 2: Seroprevalence of anti-CagA antibodies in patients with HCC and controls.

Discussion

The present study reports, for the first time, the very high seroprevalence of antibodies against the CagA protein of *H. pylori* in patients with HCC, regardless of whether or not they were infected by HCV or HBV. The prevalence was strikingly significant when pathogenic strains of the bacterium- as detected by presence of antibodies to the CagA antigen- were taken into account, but still statistically significant even for antibodies to generic strains.

A very strong correlation between HCC and *Helicobacter* infection has been demonstrated in mice experimentally infected with *H.hepaticus;* indeed, selected mouse strains rapidly develop acute and chronic hepatitis, and succumb to HCC in a very high proportion, with a strong sex predilection for males [28-31].

In humans, the oncogenic effects of *H.pylori* have been confirmed in gastric, and biliary tract neoplasias [34-37,47,48], as well as in Mucosal Associated Lymphoid Tissue (MALT) lymphomas [37]**.** A new liver carcinogen was discovered in 1994 in mice, and demonstrated to be a bacterium belonging to the *Helicobacter* species. Indeed, Ward, et al. [28] identified a new species of *Helicobacter*, known as *H.hepaticus*, in the hepatic bile canaliculi of mice, associated with HCC in chronically infected animals.

Experimental infection of male A/JCr mice with *H. hepaticus* almost inevitably leads to chronic hepatitis and HCC [28]; a number of *Helicobacter species* have been isolated from the liver of Rhesus monkeys, cats and dogs with hepatitis [29-32]. We have shown sequences belonging to the 16S rRNA of *Helicobacter* spp. in 23 of 25 livers of patients with cirrhosis and HCC [49]. Moreover, sequence analysis revealed the presence of the cagA gene in some instances in these livers. Avenaud demonstrated the presence of *Helicobacter* species in the liver of 8 out of 8 patients with HCC: *H.pylori* was the species detected in all of these livers [50]. Agha-Amiri, et al. found in the liver of seven out of 20 patients with HCC genomic sequences of a bacterium that belongs to the RNA superfamily VI (*Campylobacter*, *Helicobacter*, *Arcobacter*) [54]. Nilsson et al identified sequences of *H.pylori* and of *Helicobacter* spp. by PCR, hybridization and partial DNA sequencing, in human liver from patients with primary sclerosing cholangitis or primary biliary cirrhosis [61].

A high prevalence of *H. pylori* infection in patients with liver cirrhosis was previously reported by Siringo, et al. from Bologna [51], as they were looking for reasons for the high prevalence of duodenal ulcer in cirrhotic patients. We found a significantly higher prevalence of *H. pylori* infection in cirrhotics as compared with blood donors in Northern Italy [52,53]. Calvet, et al. found that male sex and *H. pylori* seropositivity were variables independently related to peptic ulcer in cirrhotics [62]. Chen et al found no association between peptic ulcer and *H. pylori* infection in cirrhotic patients in Taiwan [63], but Fan et al. found a higher seroprevalence of *H. pylori* in Chinese patients with HBV-related chronic hepatitis than in controls matched for age and socioeconomic status [64]. Selection biases and methodological problems with the diagnosis could be at the root of the differences in these Chinese studies. Indeed, recent studies report the strong association of *H. pylori* infection in HCC among several Chinese populations [65-67].

The association between cirrhosis and infection by *Helicobacter* spp. needs to be carefully evaluated because it might represent a novel mechanism by which some patients with HBV or HCV-related chronic liver disease succumb to malignant transformation. In Italy, only a fraction of patients infected by HBV or HCV develop liver cirrhosis, and among these only a few progress to liver cancer. Indeed, studies conducted in different areas of Italy demonstrated that 42%, 34% and 50% of the general population aged 60 years and over have circulating HCV-RNA, in Benevento,

Catanzaro and in Sicily, respectively [12-14]. Despite the high prevalence of HCV infection in these populations, only a few inhabitants have severe chronic liver disease [12-13]. A number of recent papers have reported on the long-term outcome of cohort studies in patients with HCV infection, concluding that HCV infection may be more benign than previously believed [68-74]. In Ireland, Kenny-Walsh showed that after 18 years of continuing viral carriage, cirrhosis of the liver was found in only 2% of women infected with HCV of genotype 1b by intra-venous injection of anti-RhD immunoglobulins [69].

In Germany, none of the 1500+ women similarly infected at delivery had cirrhosis after 20 years of follow-up [72] and 9% had cirrhosis after 35 years [73]. Clearly, factors beyond viral infection contribute to severity of liver illness, as we also noted in the case of HCV-associated B-cell lymphoma [75]. The role of *Helicobacter* spp. in the evolution towards cirrhosis and HCC in humans is unknown, but several potential mechanisms for liver disease due to *Helicobacter* spp. have been proposed. Taylor [76] described a new liver-specific toxin produced by several *Helicobacter* spp.; this toxin causes liver cell necrosis, followed by lympho-monocyte activation, resulting in the well-known intralobular infiltrate typical of chronic hepatitis. *H. pylori* also secretes a Polymorphonuclear (PMN) attractant protein named NAP [77], which could easily explain the accumulation of neutrophils and eosinophils observed in liver biopsies of hepatitis patients, and often attributed to the intake of excessive amount of alcoholic beverages; Furthermore, *H. pylori* itself is a source of ethanol.

Pathogenic strains of *H. pylori* encode for a protein, called CagA, which becomes phosphorylated at a tyrosine residue upon entry in human epithelial cells [78], CagA subsequently activates the ERK/MAP kinase cascade, resulting in ELK-1 phosphorylation and increased c-fos transcription [41]. Proto-oncogene activation may therefore represent a crucial step in the pathophysiology of *H. pylori*-induced neoplasia. It is therefore noteworthy that adenocarcinoma of the exocrine pancreas has been associated with high seroprevalence of *H. pylori* infection [79,80], in addition to the well-established carcinogenicity of *H. pylori* for gastric epithelial cells and for gastric mucosa lymphocytes [37,47,48,81-83].

In summary, we report for the first time that the seroprevalence of pathogenic strains of *H. pylori* in HCC patients is much more frequent than in controls, suggesting that the prognosis for HCV or HBV carriers may be influenced by co-existing or pre-existing inflammation due to *Helicobacter* spp. infection, at least in patients whose genetic background renders them susceptible to developing HCC. AP conceived the study, tested the samples and wrote the draft; TL and MFM cured and evaluated the patients; NF and EM performed the sequence analysis, statistical analysis, and contributed to the writing; TS contributed ideas and wrote. All Authors read and approved the final version

References

1. Cancer IARC (2012) Globocan, Estimated cancer incidence, mortality and prevalence worldwide in 2012. World Health Organization.
2. El-Serag HB, Mason AC (2000) Risk factors for the rising rates of primary liver cancer in the United States. Arch Intern Med 160: 3227-3230.
3. Taylor-Robinson SD, Foster GR, Arora S, Hargreaves S, Thomas HC (1997) Increase in primary liver cancer in the UK, 1979-94. Lancet 350: 1142-1143.
4. Njei B, Rotman Y, Ditah I, Lim JK (2015) Emerging trends in hepatocellular carcinoma incidence and mortality. Hepatology 61: 191-199.
5. Sherlock S and Dooley J (1997) Hepatic cirrhosis. In: Diseases of the liver and biliary system. Tenth Edition Oxford: Blackwell Scientific Publications: p371-p384.
6. De Bac C, Stroffolini T, Gaeta GB, Taliani G, Giusti G (1994) Pathogenic factors in cirrhosis with and without hepatocellular carcinoma: a multicenter Italian study. Hepatology 20: 1225-1230.
7. Villa E, Melegari M, Scaglioni PP (1991) Hepatocellular carcinoma. Risk factors others than HBV. Ital. J. Gastroenterol 23: 457-460.
8. Hayashi J, Furusyo N, Ariyama I, Sawayama Y, Etoh Y, et al. (2000) Relationship between the evolution of hepatitis C virus variants, liver damage, and hepatocellular carcinoma in patients with hepatitis C viremia. J Infect Dis 181: 1523-1527.
9. Chiaramonte M, Stroffolini T, Vian A, Stazi MA, Floreani A, et al. (1999) Rate of incidence of hepatocellular carcinoma in patients with compensated viral cirrhosis. Cancer 85: 2132-2137.
10. El-Serag HB (2011) Hepatocellular carcinoma. N Engl J Med 365: 1118-1127.
11. Yano M, Kumada H, Kage M, Ikeda K, Shimamatsu K, et al. (1996) The long-term pathological evolution of chronic hepatitis C. Hepatology 23: 1334-1340.
12. Maio G, d'Argenio P, Stroffolini T, Bozza A, Sacco L, et al. (2000) Hepatitis C virus infection and transaminase levels in the general population: a survey in a southern Italian town. J Hepatol 33:116-120.
13. Guadagnino V, Stroffolini T, Rapicetta M, Costantino A, Kondili LA, et al. (1997) Prevalence, risk factors and genotype distribution of hepatitis C virus infection in the general population: a community-based survey in southern Italy. Hepatology 26: 1006-1011.
14. Di Stefano R, Stroffolini T, Ferraro D, Usticano A, Valenza LM, et al. (2002) Endemic hepatitis C virus infection in a Sicilian town: further evidence for iatrogenic transmission. J Med Virol 67: 339-344.
15. Evangelista A, Tagliabue G, Del Sette D, Tittarelli A, Contiero P, et al. (2002) Malignant tumour follow-up in Italy, 1993-1998. IARC Sci Publ 156: 535-536.
16. Peano G, Menardi G, Ponzetto A, Fenoglio L (1994) HLA-DR5. A genetic factor influencing the outcome of hepatitis C virus infection? Arch Intern Med 154: 2733-2736.
17. Donaldson PT (1999) The interrelationship between hepatitis C virus and HLA. Eur J Clin Invest 29: 280-283.
18. Tibbs C, Donaldson P, Underhill J, Thompson L, Manabe K, et al. (1996) Evidence that HA DQA1*03 allele confers protection from chronic HCV infection in Northern caucasoids. Hepatology 24: 1342-1345.

19. Czaja AJ, Carpenter HA, Santrach PJ, Moore SB (1995) Immunologic features and HLA associations in chronic viral hepatitis. Gastroenterology 108: 157-164.

20. Alric L, Fort M, Izopet J, Vinel JP, Duffaut M, et al. (1999) Association between genes of the major histocompatibility complex class II and the outcome of hepatitis C virus infection. J Infect Dis 179: 1309-1310.

21. Vento S, Guella L, Concia E (1995) Discordant manifestations of hepatitis C in monozygotic twins. N Engl J Med 333: 1224-1225.

22. Fargion S, Mandelli C, Piperno A, Cesana B, Fracanzani AL, Fraquelli M, et al. (1992) Prognostic factors for hepatocellular carcinoma in genetic haemochromatosis. Hepatology 15: 655-659.

23. Fracanzani AL, Conte D, Fraquelli M, Taioli E, Mattioli M, et al. (2002) Increased cancer risk in a cohort of 230 patients with hereditary hemochromatosis in comparison to matched control patients with non-iron-related chronic liver disease. Hepatology 33: 647-651.

24. Kim SZ, Kupke KG, Ierardi-Curto L, Holme E, Greter J, et al. (2000) Hepatocellular carcinoma despite long-term survival in chronic tyrosinemia I. J Inherit Metab Dis 23: 791-804.

25. Eriksson S, Carlson J, Velez R (1986) Risk of cirrhosis and primary liver cancer in alpha 1-antitrypsin deficiency. N Engl J Med 314: 736-739.

26. Alpert ME, Hutt MS, Wogan GN, Davidson CS (1971) Association between aflatoxin content of food and hepatoma frequency in Uganda. Cancer 28: 253-260.

27. Uhegbu FO (1997) Dietary secondary ammines and liver hepatoma in Port Harcourt, Nigeria. Plant Foods Hum Nutr 5: 257-263.

28. Ward JM, Fox JG, Anver MR, Haines DC, George CV, et al. (1994) Chronic active hepatitis and associated liver tumors in mice caused by a persistent bacterial infection with a novel *Helicobacter* species. J Natl Cancer Inst 86: 1222-1227.

29. Burnens AP, Stanley J, Morgenstern R, Nicolet J (1994) Gastroenteritis associated with *Helicobacter pullorum*. Lancet 344: 1569-1570.

30. Fox JG, Handt L, Sheppard BJ, Xu S, Dewhirst FE, et al. (2001) Isolation of *Helicobacter cinaedi* from the colon, liver and mesenteric lymph-node of a Rhesus monkey with chronic colitis and hepatitis. J Clin Microbiol 39: 1580-1589.

31. Fox JG, Yan LL, Dewhirst FE, Paster BJ, Shames B, et al. (1995) *Helicobacter bilis* sp. nov., a novel *Helicobacter* species isolated from bile, livers, and intestine of aged, inbred mice. J. Clin. Microbiol 33: 445-454.

32. Franklin CL, Beckwith CS, Livingstone RS, Riley LK, Gibson SV, et al. (1996) Isolation of a novel *Helicobacter* species, *Helicobacter cholecystus* sp.nov., from the gallbladder of Syrian hamsters with cholangiofibrosis and centrilobular pancreatitis. J Clin Microbiol 34: 2952-2958.

33. Fox JG, Dewhirst FE, Shen Z, Feng Y, Taylor NS, et al. (1998) Hepatic *Helicobacter* species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. Gastroenterology 114: 755-763.

34. Murata H, Tsuji S, Tsujii M, Fu HY, Tanimura H, et al. (2004) *Helicobacter bilis* infection in biliary tract cancer. Aliment Pharmacol Ther 20 Suppl 1: 90-94.

35. de Martel C, M Plummer, J Parsonnet, L-J van Doorn, S Franceschi (2009) *Helicobacter* species in cancers of the gallbladder and extrahepatic biliary tract. Br J Cancer 100: 194-199.

36. Nath G, Gulati AK, Shukla VK (2010) Role of bacteria in carcinogenesis, with special reference to carcinoma of the gallbladder. World J Gastroenterol 16: 5395-5404.

37. International Agency for Research on Cancer. World Health Organization Infection with *Helicobacter pylori*. In: Schistosomes, liver flukes and *Helicobacter pylori*. Lyon: IARC; 177-202.

38. Keates S, Hitti YS, Upton M, Kelly CP (1997) *Helicobacter pylori* activates NF-kappa B in gastric epithelial cells. Gastroenterology 113: 1099-1109.

39. Maeda S, Yoshida H, Ogura K, Hirata Y, Yamaji Y, et al. (2000) *H. pylori* activates NF-kappa B through a signaling pathway involving IkB kinases, NF-kB-inducing kinases, TRAF2, and TRAF6 in gastric cancer cells. Gastroenterology 119: 97-108.

40. Mitsuno Y, Yoshida H, Maeda S, Ogura K, Hirata Y, et al. (2001) *Helicobacter pylori* induced transactivation of SRE and AP-1 through ERK signalling pathway in gastric cancer cells. Gut 49: 12-22.

41. Meyer-ter-Vehn T, Covacci A, Kist M, Pahl HL (2000) *Helicobacter pylori* activates mitogen-activated protein kinase cascades and induces expression of the proto-oncogenes c-fos and c-jun. J Biol Chem 275: 16064-16072.

42. Robles AI, Larcher F, Whalin RB, Murillas R, Richie E, G et al. (1996) Expression of cyclin D1 in epithelial tissues of transgenic mice results in epidermal hyperproliferation and severe thymic hyperplasia. Proc Natl Acad Sci (USA) 93: 7634-7638.

43. Hirata Y, Maeda S, Mitsuno Y, Akanuma M, Yamaj Y, et al. (2001) *Helicobacter pylori* activates the cyclin D1 gene through mitogen-activated protein-kinases pathway in gastric cancer cells. Infection Immunity 69: 3965-3971.

44. Barnes PJ (1997) Nuclear factor-kappa B. Int J Biochem Cell Biol 29: 867-870.

45. Baeuerle PA1, Baltimore D (1996) NF-kappa B: ten years after. Cell 87: 13-20.

46. Israel DA1, Peek RM (2001) pathogenesis of *Helicobacter pylori*-induced gastric inflammation. Aliment Pharmacol Ther 15: 1271-1290.

47. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, et al. (2000) Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 404: 398-402.

48. Figura N, Marano L, Moretti E, Ponzetto A (2016) *Helicobacter pylori* infection and gastric carcinoma: not all the strains and patients are alike. World J Gastrointest Oncol 8: 40-54.

49. Ponzetto A, Pellicano R, Leone N, Cutufia MA, Turrini F, et al. (2000) *Helicobacter* infection and cirrhosis in hepatitis C virus carriage: is it an innocent bystander or a troublemaker? Medical Hypotheses 54: 275-277.

50. Avenaud P, Marais A, Monteiro L, Lebail B, Bioulac P, et al (2000). Detection of *Helicobacter* spp in the liver of patients with and without primary liver carcinoma. Cancer 89: 1431-1439.

51. Siringo S, Vaira D, Menegatti M, Piscaglia F, Sofia S, et al. (1997) High prevalence of *Helicobacter pylori* in liver cirrhosis: relationship with clinical and endoscopic features and the risk of peptic ulcer. Dig Dis Sci 42: 2024-2030.

52. Pellicano R, Leone N, Berrutti M, Cutufia MA, Fiorentino M, et al. (2000) *Helicobacter pylori* seroprevalence in hepatitis C virus positive patients with cirrhosis. J Hepatol 33: 648-650.

53. Ponzetto A, Pellicano R, Leone N, Berrutti M, Turrini F, et al. (2000) *Helicobacter pylori* seroprevalence in cirrhotic patients with hepatitis B virus infection. Neth J Med 56: 206-210.

54. Agha-Amiri K, Meier H, Rocken C, Roessner A, Malfertheiner P (1998) New bacterial species in human hepatocellular carcinoma. Gut 43: A89.

55. Morgando A, Sanseverino P, Perotto C, Molino F, Gay V, et al. (1995) *Helicobacter pylori* seropositivity in myocardial infarction. Lancet 345: 1380.

56. Ponzetto A, La Rovere MT, Sanseverino P, Bazzoli F (1996) Association of *Helicobacter pylori* infection with coronary heart disease. Study confirms previous findings. Br Med J 312: 251.

57. Figura N, Palazzuoli A, Vaira D, Campagna MS, Moretti E, Iet al. (2014) Cross-sectional study: CagA-positive *Helicobacter pylori* infection, acute coronary artery disease and systemic levels of B-type natriuretic peptide. J Clin Pathol 67: 251-257.

58. Danielli E (1993) A fluorometric enzyme-linked immunosorbent assay for serological diagnosis of *Helicobacter pylori* infection. Eur J Gastroenterol Hepatol 5: S57-S59.

59. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R (1973) Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 60: 646-649.

60. Fox JG, Drolet R, Higgins R, Messier S, Yan L, et al. (1996) *Helicobacter canis* isolated from a dog liver with multifocal necrotizing hepatitis. J Clin Microbiol 34: 2479-2482.

61. Nilsson HO, Taneera J, Castedal M, Glatz E,Olsson R, Wadstrom T (2000) Identification of *Helicobacter pylori* and other *Helicobacter* spp by PCR, hybridization and partial DNA sequencing in human liver samples from patients with primary sclerosing cholangitis or primary biliary cirrhosis. J Clin Microbiol 38: 1072-1076.

62. Calvet X, Navarro M, Gil M, Lafont A, Sanfeliu I, et al. (1998) Epidemiology of peptic ulcer disease in cirrhotic patients: role of *Helicobacter pylori* infection. Am J Gastroenterol 93: 2501- 2507.

63. Chen JJ, Changchien CS, Tai DI, Chiou SS, Lee CM, et al. (1994) Role of *Helicobacter pylori* in cirrhotic patients with peptic ulcer. A serological study. Dig Dis Sci 39: 1565-1568.

64. Fan XG, Zou YY, Wu AH, Li TG, Hu GL, et al. (1998) Seroprevalence of *Helicobacter pylori* infection in patients with hepatitis B. Br J Biomed Sci 55: 176-178.

65. Huang Y, Fan XG, Wang ZM, Zhou JH, Tian XF, et al. (2004) Identification of *Helicobacter* species in human liver samples from patients with primary hepatocellular carcinoma. J Clin Pathol 57: 1273-1277.

66. Xuan SY, Li N, Qiang X, Zhou RR, Shi YX, et al. (2006) *Helicobacter* infection in hepatocellular carcinoma tissue. World J Gastroenterol 12: 2335-2340.

67. Wang L, Zollinger T, Zhang J (2013) Association between *Helicobacter pylori* infection and liver cancer mortality in 67 rural Chinese counties. Cancer Causes Control 24: 1331-1337.

68. Alison JR, Roberts S, Lanigan A, Bowden S, Brown T, et al. (2000) Assessment of long-term outcomes of community-acquired hepatitis C infection in a cohort with sera stored from 1971 to 1975. Hepatology 32: 582-587.

69. Kenny-Walsh E (1999) Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. N Engl J Med 340: 1228-1233.

70. Seeff LB, Miller RN, Rabkin CS, Buskell-Bales Z, Straley-Eason KD, S, et al. (2000) 45-year follow-up of hepatitis C virus infection in healthy young adults. Ann Int Med 132: 105-111.

71. Seeff LB, Buskell-Bales Z, Wright EC, Durako SJ, Alter HJ, I, et al. (1992) Long-term mortality after transfusion associated Non-A, non-B hepatitis: The National Heart, Lung and Blood Institute study. N Engl J Med 327: 1906-1911.

72. Wiese M, Berr F, Lafrenz M, Porst H, Oesen U (2000) Low frequency of cirrhosis in a hepatitis C (genotype 1b) single-source outbreak in Germany: a 20-year multicenter study. Hepatology 32: 91-96.

73. Wiese M, Fischer J, Lobermann M, Gobel U, Grungreiff K, et al. (2014) Evaluation of Liver Disease Progression in the German Hepatitis C Virus (1b)-Contaminated Anti-D Cohort at 35 Years After Infection. Hepatology 59: 49-57.

74. Thomas DL, Astemborski J, Rai RM, Anania FA, Schaeffer M, et al. (2000) The natural history of hepatitis C virus infection. Host, viral and environmental factors. JAMA 284: 450-456.

75. Ponzetto A, Carloni G2 (2016) Hepatitis C virus and lymphoma. Hepatology 64: 1813.

76. Taylor NS, Fox JG, Yan L (1995) *In-vitro* hepatotoxic factor in *Helicobacter hepaticus*, *H. pylori* and other *Helicobacter* species. J Med Microbiol 42: 48-52.

77. Satin B, Del Giudice G, Della Bianca V, Dusi S, Laudanna C, et al. (2000) The neutrophil-activating protein (HP-NAP) of *Helicobacter pylori* is a protective antigen and a major virulence factor. J Exp Med. 191: 1467-1476.

78. Stein M, Rappuoli R, Covacci A (2000) Tyrosine phosphorylation of the *Helicobacter pylori* CagA antigen after cag-driven host cell translocation. Proc Natl Acad Sci U S A 97: 1263-1268.

79. Stolzenberg-Solomon RZ, Blaser MJ, Limburg PJ, Perez-Perez G, Taylor PR, et al. (2001) *Helicobacter pylori* seropositivity as a risk factor for pancreatic cancer. J Natl Cancer Inst 93: 937-941.

80. Raderer M, Wrba F, Kornek G, Maca T, Koller DY, et al. (1998) Association between *Helicobacter pylori* infection and pancreatic cancer. Oncology 55: 16-19.

81. Wotherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, et al. (1993) Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet 342: 575-577.

82. Wang HP, Zhu YL, Shao W (2013) Role of *Helicobacter pylori* virulence factor cytotoxin-associated gene A in gastric mucosa associated lymphoid tissue lymphoma. World J Gastroenterol 19: 8219-8226.

83. Sorrentino D, Ferraccioli GB, De Vita S, Labombarda A, Avellini C, et al. (1997) Gastric B-cell clonal expansion and *Helicobacter pylori* infection in patients with autoimmune diseases and with dyspepsia. Scand J Gastroenterol 32: 1204-1208.