The Association of *Helicobacter pylori* Seropositivity with All-Cause Mortality among Colorectal Cancer Patients Undergoing PET/CT Scans

Haim Shmuely MD,5, Baruch Brenner MD2,6, David Groshar MD3,6, Nir Hadari MD4, Ofir Purim MD2,6, Meital Nidam MD3,6, Merab Eligalashvili MD3,6, Jacob Yahav MD1,5 and Hanna Bernstine MD6

1Department of Internal Medicine D and Helicobacter Research Institute, Kaplan Medical Center, Rehovot, Israel
2Institute of Oncology, Davidoff Cancer Center and 3Department of Nuclear Medicine, Rabin Medical Center (Beilinson Campus), Petah Tikva, Israel
4Dan District, Clalit Health Services, Or Yehuda, Israel
5Faculty of Medicine, Hebrew University, Jerusalem, Israel
6Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

**ABSTRACT:**

**Background:** Evidence has been emerging that *Helicobacter pylori* may also impact colorectal cancer (CRC). Positron emission tomography/computed tomography (PET/CT) imaging can predict overall survival in CRC patients.

**Objectives:** To determine a possible association between *H. pylori* seropositivity and all-cause mortality among CRC patients evaluated by PET/CT scans.

**Methods:** This prospective cohort study was comprised of 110 consecutive CRC patients who had undergone a PET/CT evaluation in a tertiary academic medical center. Data included demographics, body mass index (BMI), tumor node metastasis stage at diagnosis, treatment, time from diagnosis to PET/CT, and PET/CT findings. All patients were tested for anti-*H. pylori* immunoglobulin G (IgG) antibodies and followed for 36 months from the day of the PET/CT scan. Mortality was documented. Univariate and multivariate Cox regression was used to estimate the hazard ratio (HR) of *H. pylori* serological status.

**Results:** During the follow-up period, of the 110 CRC patients 41 (37.3%) died and 69 (62.7%) survived. Of the 41 patients, 26 (63.4%) were *H. pylori* seropositive and 15 (36.6%) were seronegative. Multivariate analysis showed that *H. pylori* seropositivity (HR 3.46, 95% confidence interval 1.63–7.32), stage IV at diagnosis, metastatic disease found on PET/CT, longer time from diagnosis to PET/CT, lower BMI, and older age were associated with increased mortality.

**Conclusions:** Our findings suggest that *H. pylori* infection may be a risk factor for all-cause mortality among CRC patients who are evaluated by PET/CT. Multicenter studies with larger patient groups are needed to confirm our findings.

**KEY WORDS:** colorectal cancer (CRC), *Helicobacter pylori*, positron emission tomography/computed tomography (PET/CT)

**H**elicobacter pylori (*H. pylori*) has been classified by the International Agency for Research on Cancer as a group 1 carcinogen and the major etiological factor for stomach cancer, the second most common cause of cancer death in the world [1].

Evidence has been emerging that *H. pylori* may also impact colorectal cancer (CRC) [2,3], one of the most commonly diagnosed cancers in the world, the third most common cancer, and the fourth most common cause of death from cancer worldwide. CRC accounts for roughly 1.2 million new cases of cancer diagnoses and 600,000 deaths per year [3]. A large study of computerized databases that reviewed surgical pathology reports confirmed this association [4]. The study, which comprised 156,000 subjects who underwent a colonoscopy and esophagogastroduodenoscopy with biopsy results from both procedures, demonstrated that *H. pylori* gastritis occurred more frequently among patients with CRC. Approximately 20% to 25% of these patients are metastatic at the time of diagnosis and nearly 50% of the remainder will develop metastases post-treatment [5]. Evaluation by positron emission tomography/computed tomography (PET/CT) imaging has proven very useful in the clinical staging and restaging of metastases or local recurrence of CRC [6]. This powerful tool can predict progression-free survival and overall survival in CRC patients [7].

The progressive decline in *H. pylori* prevalence may explain the reduction in gastric cancer mortality over the past 80 years. Since *H. pylori* is acquired almost exclusively in childhood and usually persists for a lifetime unless antimicrobial therapy is given, serum immunoglobulin G (IgG) antibodies against *H. pylori* infection are considered a valid test for long-term infection [8]. It is important to evaluate the influence of *H. pylori* seropositivity on all-cause mortality in CRC patients.

To the best of our knowledge, the association between *H. pylori* infection and mortality in CRC patients has not yet
been reported. The objective of this prospective non-interventional study was to determine the impact of \textit{H. pylori} seropositivity on mortality in CRC patients evaluated by PET/CT.

**PATIENTS AND METHODS**

**STUDY DESIGN AND POPULATION**

A prospective cohort study included all consecutive patients, older than 18 years of age with a histopathological confirmed colorectal adenocarcinoma who presented as outpatients for fluodeoxyglucose (\(^{18}\)F-FDG) PET/CT scans at the nuclear medicine department, at Rabin Medical Center from 1 February 2011 to 31 December 2011. This 900-bed university-affiliated tertiary hospital serves urban and non-urban populations and serves as a first-line and tertiary facility for approximately 1 million individuals. The most common indications for \(^{18}\)F-FDG PET/CT scans were initial staging and evaluating response to therapy. Those who agreed to participate were subsequently included in the study cohort.

Patients were excluded if they were hyperglycemic (blood glucose levels > 200 mg/dl) on the day of the PET/CT investigation, were unable to remain in a prone position, or were unable or unwilling to provide informed consent. Height and weight at study entry were recorded. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. The study was reviewed and approved by the institutional review board at the Rabin Medical Center. Informed consent was obtained from each patient. The study was supported by a research grant from the Chief Scientist’s Bureau of the Israeli Ministry of Health.

**PET/CT**

After fasting for 4 hours, all patients underwent an \(^{18}\)F-FDG PET/CT executed by a GE Discovery STE Whole Body PET/CT scanner (GE Medical Systems, Milwaukee, WI, USA). Blood glucose levels were examined before the \(^{18}\)F-FDG injection to ensure a blood glucose level < 200 mg/dl. CT images were acquired at 120 kV and 80 mA, pitch 1.75, 0.8s per tube rotation, and slice thickness of 3.75 mm. During the whole-body CT examination, 80 ml of a contrast agent (Ultravist 300, Schering AG, Berlin, Germany) was intravenously administered to ensure fully diagnostic CT data. The PET scan was performed 45–90 minutes after an intravenous injection of 10–15 mCi of \(^{18}\)F-FDG. The contrast-enhanced CT was used for attenuation correction of the PET data. The PET scan was performed from the base of the skull to the mid-thigh, 2–3 minutes per bed position, resulting in a total scan time of approximately 20–25 min (7 bed positions). An \(^{18}\)F-FDG PET/CT study without abnormal \(^{18}\)F-FDG uptake was considered as physiological or benign. An abnormal FDG uptake of the primary tumor or distant metastases was also evaluated.

**SEROLOGY**

Serum samples were obtained on the same day as the PET/CT scan and stored at -20°C until assayed. IgG antibodies against \textit{H. pylori} infection were tested by an enzyme-linked immunosorbent assay (ELISA; Orion Diagnostica, Espoo, Finland). This method was validated in our laboratory by a pilot study of patients who had undergone an endoscopy at our hospital that had yielded a sensitivity of 94%, specificity of 90%, and positive and negative predictive values of 100 and 90%, respectively.

**DATA SOURCE/MEASUREMENT**

The following data were extracted from the clinical records and recorded: demographic data, smoking status (never smoked, current smoker, former smoker), disease duration until PET/CT scan, surgery, and chemotherapy treatment in an adjuvant or metastatic setting. Surgical data included the date of surgery, pathology of the tumor, and status of any distant metastasis. These data served to determine the tumor stage at diagnosis classified according to the 7th edition of the tumor node metastasis (TNM) staging system for CRC [9] and grouped into three subgroups: early stages = I and II, locally advanced stage = III, and metastatic stage = IV. PET/CT lesion uptake was classified as: normal evaluation, colon uptake only, and metastatic disease.

**FOLLOW-UP**

All patients were followed for 36 months from the day of their PET/CT. Patient mortality was documented as recorded in the Central Population Registry (death of an Israeli resident is reported and registered in the Central Population Registry within 48 hours). Duration of follow-up was defined as the interval from the day of the PET/CT to the day of death or 36 months later.

**BIAS**

\textit{H. pylori} infection status was based only on serology, consequently not only patients with an active infection were classified as \textit{H. pylori}-positive as patients with a long-standing infection in which the bacteria had disappeared during the progression of histological alterations (i.e., atrophic gastritis) could have been included. \textit{H. pylori} seropositivity tests have high sensitivity and specificity; therefore, we believe that classification biases of the exposure were minimalized. Outcome was defined as all-cause mortality according to the Central Population Registry to avoid classification bias. Selection bias was minimized by selecting consecutive patients. Participants were offered no remuneration; thus, we believe that the selection bias was minimal.
STATISTICAL ANALYSIS

Continuous variables were observed for normal distribution using a histogram and expressed as median and interquartile range (IQR). Categorical variables were summarized as frequencies and proportions. Continuous variables were compared between seropositive and seronegative patients by the Mann–Whitney test. Differences in proportions among categorical data were assessed using the chi-squared or Fisher exact test, as appropriate. The Kaplan–Meier curve was used to describe mortality during follow-up in seropositive and seronegative patients. The log-rank test was used to compare the seropositive and seronegative patients. Univariate and multivariate Cox regression was used to evaluate the association between *H. pylori* serostatus and possible confounders with all-cause mortality. The multivariate Cox regression included 2 blocks: the Enter method, which included *H. pylori* serostatus age and gender, and the Backward Stepwise (Likelihood Ratio) method, which included all variables in the study.

Hazard ratios (HR) with a 95% confidence interval (95%CI) were reported. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 21 (IBM Corp., Armonk, NY, USA) and R version 3.2.3 (Foundation for Statistical Computing, Vienna, Austria). All statistical analyses were two tailed. P value < 0.05 was considered statistically significant. A statistical review of the study was conducted by an experienced epidemiologist.

RESULTS

A total of 110 patients, 61 males and 49 females, met the inclusion criteria, of whom 64 (58%) were *H. pylori* seropositive.

The demographic, clinical and laboratory characteristics of the entire cohort, by subgroups, with positive or negative *H. pylori* serology, are shown in Table 1. *H. pylori* seropositive and seronegative patients did not differ in age, gender, smoking status (never smoked, current smoker, former smoker), disease duration until PET/CT scan, colectomy, chemotherapy, and TNM staging at diagnosis. Seropositive patients had a lower rate of colon uptake on PET/CT.

During the 36 month follow-up period, 41 patients (37.3%) died and 69 (62.7 %) survived. Of the 41 patients who died, 26 (63.4%) were in the *H. pylori* seropositive group and 15 (36.6%) in the seronegative group (crude HR 1.39, 95%CI 0.74–2.62, P = 0.311) [Table 2]. The variables associated with increased mortality on univariate analysis were: stage IV at diagnosis, metastatic disease on PET/CT scans, and older age. Multivariate analysis demonstrated that *H. pylori* seropositivity was associated with increased mortality (HR 3.46, 95%CI 1.63–7.32, P = 0.001) [Table 2, Figure 1]. Other factors associated with higher mortality were: stage IV at diagnosis (HR 11.75, 95%CI 3.58–38.52, P < 0.001), metastatic disease found on PET/CT (HR 3.01, 95%CI 1.28–7.09, P = 0.015), longer time from diagnosis to PET/CT (HR 1.16, 95%CI 1.03–1.31, P = 0.018), lower BMI (HR 0.88, 95%CI 0.79–0.97, P = 0.012) and older age (HR 1.04, 95%CI 1.01–1.08, P = 0.005) [Table 2]. Figure 1 shows an adjusted survival curve relating to the association between *H. pylori* status and mortality.

DISCUSSION

To the best of our knowledge, our study is the first to evaluate the association of an *H. pylori* infection with all-cause mortality rates in CRC patients undergoing PET/CT scans, which is a powerful imaging tool that can predict progression-free survival and overall survival in CRC patients [7]. The most interesting finding of this prospective cohort study is the significant association between *H. pylori* seropositivity and all-cause mortality in CRC patients (HR 3.46, 95%CI 1.13–7.32, P < 0.001).

Soveri and colleagues [10] reported that functional dyspeptic symptoms associated with the presence of *H. pylori* have misled diagnostic workup in CRC patients and may lead to a diagnostic delay of CRC. Although this study did not show inferior disease-free or overall survival in *H. pylori*-positive patients compared with *H. pylori*-negative patients, the authors recommended that a diagnostic CRC workup should not be terminated due to a diagnosis of a chronic *H. pylori* infection.

In our study, *H. pylori* positive patients had a higher rate of metastatic disease associated with a higher mortality, possibly due to a delayed diagnosis. The highest mortality in the studied population was strongly associated with stage IV at diagnosis and a longer period from diagnosis to a PET/CT scan. Thus, in our CRC patients a diagnostic delay in *H. pylori* positive patients might have caused the significantly inferior outcome of

### Table 1. Clinical characteristics by *H. pylori* status in all study groups

<table>
<thead>
<tr>
<th>Entire study population</th>
<th>Positive (n=64)</th>
<th>Negative (n=46)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, IQR (median)</td>
<td>67 (59.8–73.0)</td>
<td>69.5 (65–74)</td>
<td>65 (59–73)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>61 (55.5)</td>
<td>36 (56.3)</td>
<td>25 (54.3)</td>
</tr>
<tr>
<td>Smoking history, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>74 (68.5)</td>
<td>41 (65.1)</td>
<td>33 (73.3)</td>
</tr>
<tr>
<td>Current</td>
<td>18 (16.7)</td>
<td>11 (17.5)</td>
<td>7 (15.6)</td>
</tr>
<tr>
<td>Former</td>
<td>18 (14.8)</td>
<td>11 (17.5)</td>
<td>5 (11.1)</td>
</tr>
<tr>
<td>Time, years from diagnosis to PET, IQR (median)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5 (0.3–3.6)</td>
<td>1.4 (0.3–3.5)</td>
<td>1.6 (0.4–3.7)</td>
</tr>
<tr>
<td>BMI, kg/m², IQR (median)</td>
<td>26.5 (24.7–29.1)</td>
<td>26.2 (24.7–29.2)</td>
<td>26.7 (24.6–28.0)</td>
</tr>
<tr>
<td>Chemotherapy, n (%)</td>
<td>68 (61.8)</td>
<td>37 (57.8)</td>
<td>31 (67.4)</td>
</tr>
<tr>
<td>Colectomy, n (%)</td>
<td>58 (53.2)</td>
<td>32 (50.8)</td>
<td>26 (56.5)</td>
</tr>
<tr>
<td>PET results, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal evaluation</td>
<td>48 (43.6)</td>
<td>24 (37.5)</td>
<td>24 (52.2)</td>
</tr>
<tr>
<td>Only colon uptake</td>
<td>18 (16.4)</td>
<td>16 (25.0)</td>
<td>2 (4.3)</td>
</tr>
<tr>
<td>Metastatic disease</td>
<td>44 (40.0)</td>
<td>24 (37.5)</td>
<td>20 (43.5)</td>
</tr>
<tr>
<td>Stage at diagnosis, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I–II</td>
<td>37 (33.8)</td>
<td>26 (40.6)</td>
<td>22 (23.9)</td>
</tr>
<tr>
<td>III</td>
<td>38 (34.5)</td>
<td>19 (29.7)</td>
<td>19 (41.3)</td>
</tr>
<tr>
<td>IV</td>
<td>35 (31.8)</td>
<td>19 (29.7)</td>
<td>16 (34.8)</td>
</tr>
</tbody>
</table>

BMI = body mass index, *H. pylori* = *Heliocobacter pylori*, IQR = interquartile range, PET = positron emission tomography/computed tomography
Cancer cachexia affects approximately 50% of patients with colon cancer. Across all tumor groups, cachexia is associated with a 20% mortality rate [13]. In addition, we found that older age was independently associated with mortality, as reported in other studies [14,15].

The physiopathological mechanisms underlying the association between *H. pylori* and colonic neoplasms are not completely clear. In previous studies, we [16] and others [17] demonstrated that fecal shedding of viable *H. pylori* and *H. pylori* antigens occur, under certain circumstances, and are detected in colonic tissue samples [18]. Thus, either by a direct carcinogenic effect of the bacteria on the colorectal mucosa or an indirect action through hypergastrinemia, a trophic effect on the mucosal cells of the colon is produced [19].

Gastrin has been implicated in the tumor genesis of the gastrointestinal tract as a growth factor, increasing the tumor’s spread and angiogenesis. It can also impart anti-apoptotic properties, thus activating the transcription of factors involved in the transformation of colon adenomas into malignant carcinomas [20]. The association between *H. pylori* and elevated gastrin levels has been well established [21].

**STUDY STRENGTHS AND LIMITATIONS**

Despite the demonstration of a strong prognostic significance of *H. pylori* status, our study has several potential limitations. First, the follow-up duration was relatively short. However, other than a 3 year follow-up, the patients in this study also had...
a median time of 1.5 years from diagnosis to PET/CT. Second, this was a hospital-based study, hence our patients may not have been sufficiently representative of the general population in Israel. The study was performed in central Israel where a PET/CT scan is an accessible diagnostic modality. Moreover, our study did not include measurements of potential mediators of H. pylori-related risks, such as gastrin levels and atrophic gastritis. Despite these limitations, our study seems to have clinical relevance, as to the best of our knowledge, it is the first report investigating the relationship between H. pylori status and prognosis in CRC patients with a 3 year follow-up. Large prospective studies with additional measurements of such mediators would be desirable.

CONCLUSIONS
Seropositivity for H. pylori antibodies found in CRC patients evaluated by PET/CT scans may be significantly associated with increased all-cause mortality. A diagnostic delay in H. pylori positive patients might have caused the significantly inferior outcome of this group. Investigating the mechanisms by which H. pylori may increase all-cause mortality in these patients is essential to our comprehensive understanding of the biology of both H. pylori and CRC.

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Correspondence
Dr. H. Shmuely
Dept. of Internal Medicine D, Kaplan Medical Center, Rehovot 76100, Israel
Phone: (972-8) 944-1866
Fax: (972-8) 944-1866
email: hshmuely@zahav.net.il

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