Relationship between *Helicobacter Pylori* Infection and Risk of Metabolic Dysfunction-Associated Steatotic Liver Disease: An Updated Meta-analysis

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LIST OF ABBREVIATIONS
NAFLD, non-alcoholic fatty liver disease
MASLD, metabolic dysfunction-associated steatotic liver disease
H. pylori, Helicobacter pylori
PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses
NOS, Newcastle-Ottawa Quality Assessment Scale
T2DM, type 2 diabetes mellitus

ABSTRACT

Background: Recent observational studies examining the association between *Helicobacter pylori* infection and the risk of metabolic dysfunction-associated steatotic liver disease (MASLD) have reported conflicting results. We performed a meta-analysis to quantify the magnitude of the association between *H. pylori* infection and the risk of MASLD.

Methods: We systematically searched three large electronic databases to identify eligible observational studies (published up to November 30, 2023) in which liver biopsy, imaging or blood-based biomarkers/scores were used for diagnosing MASLD. Data from selected studies were extracted and meta-analysis was performed using common and random-effects modeling. Statistical heterogeneity among published studies, subgroup analyses, meta-regression analyses, and publication bias were assessed.

Results: A total of 28 observational studies (24 cross-sectional and 4 longitudinal studies) were identified, including 231,291 middle-aged individuals of predominantly Asian ethnicity (~95%). Meta-analysis of cross-sectional studies showed that *H. pylori* infection was associated with a small increase in risk of prevalent MASLD (n=24 studies; random-effects odds ratio 1.11, 95% CI 1.05-1.18; I^2=63%). Meta-analysis of data from longitudinal studies showed that *H. pylori* infection was significantly associated with an increased risk of developing incident MASLD over a mean 5-year follow-up (n=4 studies; random-effects odds ratio 1.20, 95%CI 1.08-1.33; I^2=44%). Sensitivity analyses did not modify these results. Funnel plot did not reveal any significant publication bias.

Conclusions: *H. pylori* infection is associated with a mildly increased risk of prevalent and incident MASLD. Further well-designed prospective and mechanistic studies are required to better decipher the link between *H. pylori* infection and the risk of MASLD.

Keywords: nonalcoholic fatty liver disease; NAFLD; metabolic dysfunction-associated steatotic liver disease; MASLD; Helicobacter pylori; H. pylori

LAY SUMMARY

This meta-analysis of observational studies shows that *Helicobacter pylori* infection is associated with an increased risk of having or developing metabolic dysfunction-associated steatotic liver disease (MASLD). Further well-designed prospective and mechanistic studies are needed to better elucidate the possible link between *H. pylori* infection and the increased risk of MASLD.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease worldwide [1]. The global prevalence of NAFLD in adults is estimated to be ~30% in the general population [2], up to nearly 65% in patients with type 2 diabetes mellitus (T2DM) [3], and ~80% in individuals with obesity [4]. NAFLD is closely linked with obesity, insulin resistance, metabolic syndrome, and T2DM [1]. In 2023, three large multinational liver associations proposed a nomenclature change from NAFLD to metabolic dysfunction-associated steatotic liver disease (MASLD) [5]. Increasing evidence shows an excellent concordance rate between NAFLD and MASLD definitions — i.e., with ~95-99% of individuals with NAFLD meeting MASLD criteria and, therefore, both definitions have superimposable natural histories [6-8]. In the past decade, it has become increasingly evident that MASLD is a "multisystem disease" [1, 9], where insulin resistance and related metabolic dysfunction play a pathogenic role in the development of MASLD and its major liver-related and extrahepatic outcomes, such as cardiovascular disease [10, 11], chronic kidney disease [12], and certain extrahepatic cancers, especially gastrointestinal cancers [13, 14].

Helicobacter pylori (*H. pylori*) infection is a major health problem worldwide, causing considerable morbidity and mortality [15]. Conservative estimates indicate that *H. pylori* infects more than half of the world's population, with the highest prevalence rates in Asian countries [16]. *H. pylori* infection is an established risk factor for peptic ulcer disease, non-cardia gastric cancer and gastric mucosa-associated lymphoid lymphoma that it directly promotes by inducing gastric chronic inflammation [17]. *H. pylori* is also associated with an increased risk of some extra-gastric manifestations, such as cardiovascular, neurological, hematologic, and metabolic diseases [18].

In recent years, the link between *H. pylori* infection and the risk of MASLD has also attracted considerable scientific interest. As discussed below, there have been many observational studies of the effect of *H. pylori* infection on the risk of MASLD, but these have produced conflicting results. Thus, the association between *H. pylori* infection and MASLD remains unclear.

Consequently, we undertook a comprehensive meta-analysis of observational studies with meta-regression to investigate whether there was effect-modification by features of metabolic dysfunction (e.g., type 2 diabetes mellitus (T2DM), obesity or hypertension) and also by age and sex,

in order to provide a quantitative estimate of the magnitude of the association between *H. pylori* infection and the risk of MASLD in adult individuals from different countries.

MATERIALS AND METHODS

Registration of review protocol

The systematic review protocol was registered in advance on the Open Science Framework (OSF) database (registration DOI: https://doi.org/10.17605/OSF.IO/WBZA5). The protocol has been performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [19] and the Meta-analysis Of Observational Studies in Epidemiology (MOSE) guidelines [20].

Data sources and search strategy

We systematically searched PubMed, Scopus, and Web of Science from database inception to November 30, 2023, to identify eligible observational studies examining the risk of prevalent and incident MASLD amongst adult (age ≥18 years) individuals with and without *H. pylori* infection. The search-free text terms were "nonalcoholic fatty liver disease" (OR "fatty liver" OR "NAFLD" OR "non-alcoholic steatohepatitis" OR "metabolic dysfunction-associated fatty liver disease" OR "MAFLD" OR "metabolic dysfunction-associated steatotic liver disease" OR "MASLD") AND "*Helicobacter pylori* infection" OR "*Helicobacter pylori*" OR "*Helicobacter* infection" OR "*H. pylori*". Searches were restricted to human studies, and no language restriction was imposed. We also reviewed references from relevant original papers and review articles to identify additional eligible studies not covered by the original database searches.

Inclusion and exclusion criteria

Eligible studies were included in the meta-analysis if they met the following inclusion criteria: 1) observational cross-sectional and longitudinal studies examining the association between *H. pylori* infection and risk of MASLD (or NAFLD); 2) studies reporting odds ratios (OR) or hazard ratios (HR) with corresponding 95% confidence intervals (95% CI) values for the exposure/outcome of interest; 3) studies with sufficient data for the exposure/outcome of interest to calculate unadjusted ORs or unadjusted HRs; 4) studies in which the diagnosis of MASLD (or NAFLD) was based on liver biopsy, imaging methods (mainly liver ultrasonography) or blood-based markers/scores for MASLD (i.e.,

hepatic steatosis index, fatty liver index or NAFLD-liver fibrosis score); and 5) studies in which the presence or previous exposure to *H. pylori* infection was diagnosed using invasive tests (endoscopic gastric biopsy) or non-invasive tests, such as serological testing (i.e., specific *H. pylori* IgG enzyme-linked immunosorbent assays), ^{13}C-labeled or ^{14}C-labeled urea breath tests or fecal antigen tests. The major exclusion criteria of the meta-analysis were as follows: 1) studies published as theses, congress abstracts, case reports, reviews, practice guidelines, commentaries, or editorials; 2) studies that did not exclude individuals with significant alcohol consumption (usually defined as <20 g/day for women and <30 g/day for men) or other competing causes of chronic liver disease (e.g., viral hepatitis, iron overload and use of potentially hepatotoxic drugs); and 3) studies performed in the pediatric population (<18 years).

Two investigators (MGL and NB) independently reviewed the titles and abstracts of all studies initially identified using the abovementioned inclusion criteria. Each study meeting the requirements of the first-round inclusion criteria then underwent a full-text independent review by both investigators. Eventual disagreements between investigators about the inclusion of eligible studies were resolved by a third investigator (AM).

Data extraction and quality assessment

For each eligible study, we extracted data on study design, sample size, publication year, study country, subjects' characteristics, methods used for the diagnosis of both MASLD and *H. pylori* infection, outcome of interest, list of covariates adjusted in multivariable regression analyses, and follow-up duration (only for longitudinal studies). In the case of multiple publications of the same database, we included the most up-to-date or comprehensive information.

Two investigators (MGL and NB) independently assessed the risk of bias. Any discrepancies were addressed by a re-evaluation of original articles by a third author (AM). For each study included in meta-analyses, the quality assessment was performed according to the Newcastle-Ottawa Quality Assessment Scale (NOS) [21]. We used a NOS scale adapted for cross-sectional studies [21]. Briefly, the NOS scale uses a star system to assess the quality of a study in three domains: selection, comparability, and outcome/exposure. The NOS assigns a maximum of five stars for selection (or four stars in the case of longitudinal studies), two for comparability, and three for

outcome/exposure. We judged studies that received a NOS score of at least eight stars to be at low risk of bias, thus indicating the highest quality.

Data synthesis and analysis

The outcome of interest of the meta-analysis was the presence or the development of new cases of MASLD. The ORs (for cross-sectional studies) or HRs (for longitudinal studies) and the corresponding 95% CIs were considered as the effect size (ES) for all the eligible studies. When studies had multiple adjusted regression models, we extracted only those values reflecting the maximum extent of adjustment for known risk factors and potential confounders. The OR/HRs of the eligible studies were pooled, and then an overall ES estimate was calculated using common and random-effects models.

A visual inspection of the forest plots was used to examine the possibility of statistical heterogeneity [22]. The statistical heterogeneity among studies was assessed by the chi-square test and the I^2 statistic, which estimates the percentage of variability across studies due to heterogeneity rather than chance alone [23]. The proportion of heterogeneity accounted for by between-study variability was assessed using the I^2-statistic and adjudicated to be significant if I^2-index was >50% [23].

To examine the possible sources of heterogeneity among the eligible studies and test the robustness of the associations, we conducted subgroup analyses stratifying the eligible studies by study country, diagnostic methods used for diagnosing MASLD and *H. pylori* infection, degree of statistical covariate adjustment and overall quality by NOS scale. We also tested for the possibly excessive influence of individual studies using a meta-analysis influence test that eliminated each included study one at a time. Finally, we performed univariable meta-regression analyses to test the association of MASLD with age, sex, body mass index (BMI), percentage of hypertension or pre-existing T2DM. These analyses were not performed if the total number of studies included was less than 10 [22].

The possibility of publication bias was evaluated using the funnel plot and Begg's rank correlation test; the trim and fill method was also used to further examine the possibility of publication bias [24]. We used R software (version 4.2.2/2022) for all statistical analyses with the following packages: *meta* and *metafor*.

RESULTS

Supplementary Figure 1 shows the PRISMA flow diagram of the meta-analysis. After excluding duplicates, we identified 34 potentially eligible studies from three large electronic databases from the inception to November 30, 2023. After examining the full text of these publications, we further excluded six studies [25-30], because of unsatisfactory inclusion criteria or unsatisfactory outcome measures (**Supplementary Table 1**).

The main characteristics of the observational studies included in the meta-analysis are summarized in **Table 1** for cross-sectional studies and **Table 2** for longitudinal studies. Twenty-four studies [31-54] had a cross-sectional design, whereas four studies [55-58] had a longitudinal design. Regarding the cross-sectional studies (**Table 1**), 12 studies were carried out in China, two in Japan, two in the USA, two in South Korea, one in Austria, one in Bangladesh, one in Egypt, one in Greece, one in Guatemala, and one in Iran, respectively. MASLD was diagnosed by ultrasonography in 17 of these studies, by vibration-controlled transient elastography (Fibroscan® with controlled attenuation parameter [CAP]) in three studies, by the hepatic steatosis index (HSI) or other blood-based scores (fatty liver index or NAFLD-liver fat score) in two studies, and by liver biopsy in the remaining two studies. *H. pylori* infection was diagnosed by urea breath tests in 14 studies, by blood antibodies in seven studies, by blood antibodies and/or fecal antigen tests in one study, by endoscopic gastric biopsy in one study, and by endoscopic gastric biopsy with or without fecal antigen test in one study. Only a few studies measured serum *H. pylori* anti-CagA (cytotoxin-associated gene A antigen) and/or anti-VacA (vacuolating cytotoxin A) antibodies. Ten of these 24 cross-sectional studies obtained more than eight stars on the NOS scale, thus reflecting a low risk of bias.

Regarding the four retrospective longitudinal studies included in the meta-analysis (**Table 2**), one study was carried out in China, one in Egypt and two in South Korea. MASLD was diagnosed by ultrasonography in three of these studies and by blood-based scores in one study. *H. pylori* infection was detected by blood antibodies in two studies, fecal antigen tests in one study, and urea breath tests in one study. Half of these studies obtained at least eight stars on the NOS scale, thus reflecting a low risk of bias.

H. pylori infection and risk of prevalent MASLD

Figure 1 shows the forest plot and pooled estimates of the effect of *H. pylori* infection on the risk of MASLD in 24 cross-sectional studies [31-54]. Overall, these studies included a total of 209,439 middle-aged individuals (58% men; mean age 49±8 years; mean BMI 25.3±2.4 kg/m^2; ~95% from Asian countries), 27.5% (n=62,628) of whom had a diagnosis of MASLD and 38.2% had a diagnosis of prior or active *H. pylori* infection. We found that *H. pylori* infection was significantly associated with a small increase in the risk of prevalent MASLD (pooled random-effects OR 1.11, 95%CI 1.05-1.18; I^2=63%).

Stratifying these cross-sectional studies by country, the association between *H. pylori* infection and risk of MASLD was significant in Asian studies (n=19 studies; random-effects OR 1.11, 95%CI 1.05–1.17; I^2=64%), but not in those conducted in other countries (n=5; random-effects OR 1.48, 95%CI 0.92–2.38; I^2=66%) (**Supplementary Figure 2**). Stratifying the studies by MASLD diagnosis, the association between *H. pylori* infection and the risk of MASLD was significant in studies in which MASLD was detected by liver biopsy (n=2; random-effects OR 3.15, 95%CI 1.48–6.70; I^2=0%) or ultrasonography (n=17; random-effects OR 1.10, 95%CI 1.03–1.18; I^2=65%) (**Supplementary Figure 3**). Stratifying the studies by methods used for diagnosing *H. pylori* infection, the association between *H. pylori* infection and the risk of MASLD was significant in studies where *H. pylori* infection was detected by urea breath tests (n=14; random-effects OR 1.12, 95%CI 1.05–1.20; I^2=64%) and by endoscopic gastric biopsy and/or blood antibodies (n=1; random-effects OR 4.27, 95%CI 1.26–14.5; I^2=not determined), but not in studies where *H. pylori* infection was detected by blood antibodies (n=7; random-effects OR 1.08, 95%CI 0.86–1.36; I^2=65%), by blood antibody with fecal antigen tests (n=1; random-effects OR 1.70, 95%CI 0.79–3.65), or by endoscopic gastric biopsy only (n=1; random-effects OR 0.96, 95%CI 0.82–1.13) (**Supplementary Figure 4**). Stratifying the studies by degree of covariate adjustments, the association between *H. pylori* infection and risk of MASLD was significant both in studies with varying degrees of covariate adjustments (n=15; random-effects OR 1.12, 95%CI 1.04-1.22; I^2=67%) and in those with no adjustment (n=9; random-effects OR 1.09, 95% CI 1.02-1.16; I^2=58%) (**Supplementary Figure 5**). Finally, stratifying the studies by NOS scale, the association between *H. pylori* infection and the risk of MASLD was significant both in studies with NOS <8 (n=14; random-effects OR 1.27, 95% CI 1.01-1.58; I^2=72%) and in those with NOS ≥8 (n=10; random-effects OR 1.13, 95% CI 1.04-1.21; I^2=31%) (**Supplementary Figure 6**).

Only a few cross-sectional studies tested the association between *H. pylori* infection and the risk of advanced MASLD. Therefore, we decided not to perform a formal meta-analysis to test the association between *H. pylori* infection and the risk of more advanced liver disease in MASLD. Wang *et al.* reported that *H. pylori* infection was not associated with higher ultrasonographic scores of steatosis [52]. In a subgroup of patients undergoing liver transient elastography (Fibroscan® with CAP), Wernly *et al.* showed that *H. pylori*-positive patients had higher grades of CAP-assessed hepatic steatosis than *H. pylori*-negative patients, but no significant differences were found in liver stiffness measurements (LSMs) on Fibroscan® [51]. In the study by Abo-Amer *et al., H. pylori* infection was associated with increased degrees of steatosis on Fibroscan®-assessed CAP but not with greater Fibroscan®-measured LSMs [46]. Similar results were reported by Fialho *et al*. [54]). Overall, these few cross-sectional studies suggest that there is a positive, graded association of prior or active *H. pylori* infection with the degree of hepatic steatosis but not with liver stiffness measurements in MASLD, suggesting that *H. pylori* infection might contribute to the pathogenesis of MASLD, though not to its progression to steatohepatitis and advanced fibrosis.

Sensitivity analyses and meta-regressions

A sensitivity analysis using the one-study remove (leave-one-out) approach to test the influence of each study on the overall effect size showed that the exclusion of one study at a time did not have any effect on the significant association between *H. pylori* infection and risk of MASLD (**Supplementary Figure 7**). **Supplementary Figure 8** shows the forest plot and pooled estimates of the effect of *H. pylori* infection on the risk of MASLD in the eligible cross-sectional studies, stratified by the use of blood antibodies alone (n=7; random-effects OR 1.08, 95%CI 0.86-1.36; I^2=65%) *vs*. all other methods for diagnosing *H. pylori* infection (n=17; random-effects OR 1.12, 95%CI 1.05-1.20; I^2=64%). Univariable meta-regressions showed the lack of significant effects of age (**Supplementary Figure 9**), sex (**Supplementary Figure 10**), BMI (**Supplementary Figure 11**), and pre-existing T2DM (**Supplementary Figure 12**) or hypertension (**Supplementary Figure 13)** on the association between *H. pylori* infection and risk of prevalent MASLD.

H. pylori infection and risk of incident MASLD

Figure 2 shows the forest plot and pooled estimates of the effect of *H. pylori* infection on the risk of developing MASLD in four retrospective Asian cohort studies [55-58]. These studies included a total of 21,852 individuals (52% men; mean age 51±4 years; mean BMI 23±1.1 kg/m^2), 50.5% of whom

had a diagnosis of prior or active *H. pylori* infection at the baseline, and 4,808 cases developed incident MASLD over a mean follow-up period of 5 years. The figure shows that *H. pylori* infection was associated with a higher risk of developing MASLD (pooled random-effects HR 1.20, 95%CI 1.08-1.33; I^2=44%). A sensitivity analysis using the one-study remove approach did not show any relevant effect of the exclusion of each study at a time on the significant association between *H. pylori* infection and the risk of developing MASLD (**Supplementary Figure 14**). Due to the (relatively) low between-study heterogeneity of cohort studies, we did not perform subgroup analyses for these studies.

Publication bias

As shown in **Supplementary Figure 15**, the Begg's rank correlation test did not reveal any statistically significant asymmetry of the funnel plot (p=0.063), thus suggesting that the publication bias was unlikely. These results were also confirmed by the trim and fill method (data not shown).

DISCUSSION

The main and novel findings of this comprehensive meta-analysis that included 28 observational studies (24 cross-sectional [31-54] and 4 longitudinal studies [55-58]) for a total of 231,291 middle-aged individuals of predominantly Asian ethnicity are as follows: (*i*) *H. pylori* infection was significantly associated with a robust small increase in prevalence of MASLD (detected by liver biopsy, imaging, or blood-based biomarkers/scores); (*ii*) *H. pylori* infection was significantly associated with a mildly increased risk of incident MASLD over a mean 5-year follow-up; (*iii*) these results remained significant in those studies where statistical analysis was adjusted for age, sex, adiposity measures, pre-existing T2DM, hypertension and other potential confounders, or even after stratifying by NOS scale; (*iv*) meta-regression analyses showed no significant effects of age, sex, BMI, hypertension or pre-existing T2DM on the association between *H. pylori* infection and risk of MASLD in cross-sectional studies; (*v*) subgroup analyses of cross-sectional studies showed that the association between *H. pylori* infection and MASLD risk was significant in Asian studies (but not in studies conducted in other countries outside Asia); and finally (*vi*) the association between *H. pylori* infection and the risk of MASLD was significant in studies where *H. pylori* infection was detected by urea breath tests and by endoscopic gastric biopsies, but not in those where *H. pylori*

infection was detected by blood antibody tests alone. This latter finding could be largely expected. Indeed, serologic testing for *H. pylori* IgG is no longer recommended for diagnosing active *H. pylori* infection in areas where the prevalence is less than 30%. Because blood antibodies may persist for several years, serologic testing for *H. pylori* IgG has a specificity of less than 80% for active *H. pylori* infection [59, 60].

To our knowledge, this is the most updated and comprehensive meta-analysis examining the association between *H. pylori* infection and the risk of having or developing MASLD. From 2018 to 2022, some systematic reviews and meta-analyses with fewer observational studies included [61-65] have been undertaken. In 2023, Liu *et al*. [66] meta-analyzing 28 studies (24 cross-sectional, 2 case-control, and 2 cohort studies altogether) reported that *H. pylori* infection was associated with a mildly increased risk of MASLD (pooled OR 1.27; 95%CI 1.18-1.38), although a highly significant heterogeneity was observed (I^2=89.7%). More recently, Xu *et al.* [67] performed a meta-analysis of 27 cross-sectional studies, 3 case-control studies, and 4 cohort studies. Similarly to our meta-analysis, Xu *et al.* showed that *H. pylori* infection was significantly associated with the presence of NAFLD (pooled unadjusted OR 1.26, 95% CI 1.17–1.36; I^2=89%) and the risk of incident NAFLD (pooled adjusted HR 1.18, 95% CI 1.05-1.34; I^2=71%) [67]. In contrast to the meta-analysis by Xu *et al.* [67], we performed extensive meta-regressions analyses to examine whether there was effect-modification by age, sex, T2DM and other metabolic syndrome features (obesity and hypertension) on the association between *H. pylori* infection and risk of MASLD. Furthermore, we excluded the study by Doulberis *et al.* [29], because it had a reduced statistical power (characterized by large 95% CIs). Finally, contrary to the meta-analysis by Xu *et al.*, we did not include "grey" literature, as we excluded unpublished studies and studies published outside widely available journals. There is debate about the inclusion or exclusion of 'grey' literature in a meta-analysis. The main reasons for reluctance to include "grey" literature include the absence of peer-review of unpublished literature. We believe that excluding "grey" literaturecontributes to include high-quality studies for performing a meta-analysis and provides a more accurate magnitude of the association between *H. pylori* infection and risk of MASLD. Consequently, in our meta-analysis, we observed a lower heterogeneity of the pooled primary analyses of both cross-sectional (I^2=63% *vs*. 89%) and longitudinal studies (I^2=44% *vs*. 71%) compared to that observed in the meta-analysis of Xu *et al.* That said, although a relatively high heterogeneity is more likely for meta-analyses of observational studies than for meta-analyses of randomized clinical trials when we examined the possible sources of heterogeneity, we

found that the medium-high heterogeneity observed for the cross-sectional studies was mainly due to differences in study country and methods used for diagnosing *H. pylori* infection and MASLD.

Collectively, the results of our meta-analysis support the conclusion that *H. pylori* infection is significantly associated with a robust small increase in the risk of prevalent and incident of MASLD, the evidence being stronger in Asian individuals. However, the observational design of the studies included in the meta-analysis does not allow us to draw any definitive causal inferences. From the few cross-sectional studies available, there is a positive, graded relationship between *H. pylori* infection and the severity of hepatic steatosis, but it remains uncertain whether *H. pylori* infection may also affect steatohepatitis and liver fibrosis in MASLD. A recent Mendelian randomization study revealed no genetic evidence for a causal relationship between *H. pylori* infection and MASLD [27]. Hence, routine screening for MASLD in patients positive for *H. pylori* infection might seem poorly supported by current evidence. However, it is important to note that the Mendelian randomization study mentioned above had some important limitations. First, the diagnosis of *H. pylori* infection was based solely on serological tests in the genome-wide association studies (GWAS) data, which may have affected the detection of *H. pylori* infection. Second, the dataset included only European populations. Although using a single European population to investigate the causal relationship may minimize population stratification bias, it may not be generalizable to other non-European populations [27]. Third, the study relied on a very limited number (two) of genetic instruments predisposing to *H. pylori* infection by modulating the immune response, thereby having a high likelihood of pleiotropic effects on the risk of MASLD. In addition, the scenario may be more intricate since some intervention studies suggest that *H. pylori* eradication therapies could exert some beneficial effects on insulin resistance, serum liver enzymes and other surrogate markers of MASLD [25, 68]. With this caveat in mind, we believe that the results of the eligible retrospective cohort studies reporting a significant association between *H. pylori* infection and the risk of developing MASLD should be interpreted cautiously due to the retrospective design of the studies. Furthermore, some of these Asian cohort studies included patients with different *H. pylori* eradication status at the baseline, and most importantly, no detailed information was available in these studies on the use of *H. pylori* eradication therapies over the follow-up. Therefore, we cannot know if and how these results may have been influenced by *H. pylori* eradication therapy.

Identifying the potential pathophysiological mechanisms underpinning the association between *H. pylori* infection and the risk of MASLD is beyond the scope of this meta-analysis. A recent review deeply discussed the possible links between *H. pylori* infection and MASLD [69]. That said, more than 15 years ago, some investigators reported the presence of *Helicobacter* species in liver samples of patients with various chronic liver diseases, including MASLD [68, 70, 71]. Recent studies have shown that *H. pylori* infection may promote the development and progression of MASLD, possibly through several mechanisms, such as low-grade inflammation, increased oxidative stress, insulin resistance, and alterations of gut permeability [68]. In particular, low-grade inflammation in the gastric mucosa may exacerbate and promote the local and systemic release of several proinflammatory cytokines, thereby exacerbating systemic insulin resistance, increasing gut permeability and altering the gut microbiome composition [68]. *H. pylori* infection may alter *Bacteroidetes, Lactobacillus, Proteobacteria, Firmicutes* and *Actinobacteria* colonies, thus changing the gut microbiota composition like that observed in obese patients [68]. Furthermore, it has been suggested that *H. pylori* infection might also act as a cofactor that interacts with coexisting metabolic risk factors in the pathogenesis of MASLD [72]. *H. pylori* infection might also be, at least in part, implicated in the association between MASLD and the increased risk of developing incident gastric cancers [13, 73].

Our meta-analysis has some important limitations strictly inherent to the included studies. First, only four of the 28 included observational studies had a prospective design. Second, although we used a random-effects model, the interpretation of some meta-analysis results requires some caution because of the observed medium-high heterogeneity for the cross-sectional studies. Third, due to the incomplete covariate adjustment in most studies, the possibility of residual confounding by unmeasured factors cannot be entirely excluded. Fourth, most studies were performed in Asian countries. Since the Asian and non-Asian populations have distinct genetic backgrounds, body fat distributions, dietary habits, and different rates of *H. pylori* infection, additional studies are needed to better elucidate the relationship between *H. pylori* infection and the risk of MASLD in non-Asian people. Fifth, most studies diagnosed MASLD using imaging methods (principally ultrasonography), but only a few studies used liver biopsy (i.e., the gold standard) for diagnosing and staging MASLD. Similarly, the diagnostic approaches used for *H. pylori* infection were different. Most studies used urea breath tests, blood antibodies or fecal antigen tests to diagnose *H. pylori* infection, whereas only two studies confirmed the diagnosis by endoscopic gastric biopsy examination (i.e., the gold

standard method for diagnosing *H. pylori* infection). According to the recent Maastricht VI/Florence Consensus Report, urea breath tests and stool antigen tests are the best recommended non-invasive diagnostic tests for active *H. pylori* infection, while serological antibody tests alone are less accurate [74]. In the few cross-sectional studies that measured serum anti-CagA antibodies and examined the influence of CagA (i.e., the virulence factor of *H. pylori*) on the risk of MASLD, the authors found that only CagA-negative *H. pylori* positivity was significantly associated with the presence of MASLD after adjustment for potential confounders, confirming that an association exists between these two conditions [38]. However, further research is needed to better understand whether gastric metaplasia or dysplasia related to the severity of *H. pylori* infection may be associated with a higher risk of MASLD. Finally, although all eligible studies have used the NAFLD nomenclature, for the purpose of this meta-analysis, we have assumed that, as the two fatty liver disease definitions are largely overlapping, the increase in the risk conferred by *H. pylori* infection was comparable for NAFLD and MASLD, which should be confirmed in future studies.

Despite these limitations, our meta-analysis also has important strengths. This meta-analysis provides the most comprehensive and updated assessment of the nature and magnitude of the association between *H. pylori* infection and the risk of MASLD. Moreover, we have used standardized risk estimates from all included studies to allow a consistent combination of estimates across studies. The overall quality of the studies included in the meta-analysis appears to be relatively moderate, with a relatively low risk of bias according to the NOS scale. The large number of individuals with MASLD has also provided high statistical power to quantitatively assess the association between *H. pylori* infection and the risk of MASLD. Finally, although a selective reporting bias of eligible studies could not be excluded (because we did not include “grey” literature in the meta-analysis), we think our comprehensive search has made it unlikely that any published report was missed. At the same time, visual inspection of the funnel plot and formal statistical tests did not show any significant publication bias.

In conclusion, our updated meta-analysis of observational studies showed that *H. pylori* infection was associated with a mildly increased risk of prevalent and incident MASLD in middle-aged individuals from different countries. This association remained significant even after adjustment for age, sex, adiposity measures, T2DM and other known risk factors for MASLD. Meta-regressions showed the lack of any significant effects of age, sex, BMI, and prevalence of pre-existing T2DM or

hypertension on the association between *H. pylori* infection and the risk of MASLD. Further well-designed studies, especially in non-Asian populations, are required to corroborate these findings. Further investigations are also needed to examine whether *H. pylori* eradication therapies may prevent or improve MASLD development.

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Data availability statement: All supporting data of the meta-analysis are available within the article (and in the online-only data supplement).

Author Contributions: AM and GT were involved in the conception, design, and conduct of the study, the analysis and interpretation of the results and wrote the first draft of the manuscript. MGL, NB, ES and BP searched the published articles. FG, ZB, LV, CDB and HT contributed to the discussion. All authors edited, reviewed, and approved the final version of the manuscript.

Table 1. Main characteristics of eligible cross-sectional studies (n=24) assessing the association between prior or active *H. pylori* infection and the risk of prevalent MASLD.

Author, Ref	Year	Country	Sample size (n)	Age (years)	BMI (kg/m^2)	Percentage of known T2DM (%)	Percentage of men (%)	MASLD diagnosis (% of MASLD)	*H. pylori* infection diagnosis (% of HP positivity)	Covariate adjustment	Odd ratios (95% CI)	NOS scale
Polyzos et al. [31]	2013	Greece	53	54	31	NA	22.6%	Biopsy (52.8%)	History of HP eradication treatment and/or blood antibodies or urea breath test (69.8%)	Age, sex, BMI, insulin resistance	3.61 (1.04-12.6)	6
Sumida et al. [32]	2015	Japan	130	55	27.5	70%	50%	Biopsy (100%)	Blood antibodies (40%)	Age, sex, obesity, dyslipidemia, hypertension, T2DM	2.91 (1.11-7.6)	5
Okushin et al. [33]	2015	Japan	5,289	49	23	NA	34.3%	Ultrasound (34.1%)	Blood antibodies (27.4%)	Unadjusted	1.13 (0.99-1.28)	7
Zhang et al. [34]	2016	China	1,200	NA	NA	NA	0%	Ultrasound (50%)	Urea Breath test (37%)	Age, sex, race, birthplace	3.17 (1.91-5.74)	6
Baeg et al. [35]	2016	South Korea	3,663	53	23.6	12%	58.4%	HSI >36 (or NAFLD-LFS > -0.640) (25.8%)	Urea Breath test (44.7%)	Age, sex, smoking, hs-CRP	1.13 (0.97-1.31)	6
Chen CX et al. [36]	2017	China	2,263	69	NA	NA	62.9%	Ultrasound (26.6%)	Urea Breath test (45.8%)	Age, sex, BMI, waist circumference, HbA1c, triglycerides, uric acid, transaminases	1.39 (1.05-1.73)	8
Cai et al. [37]	2018	China	2,051	38	23.5	NA	34.8%	Ultrasound (21.1%)	Urea Breath test 31.4%)	Sex, BMI, fasting glucose, triglycerides, HDL-cholesterol	0.94 (0.70-1.27)	8
Kang et al. [38]	2018	USA	5,404	44	27	4.8%	47%	Ultrasound (30.8%)	Blood antibodies and anti-CagA IgG antibodies (49.1%)	Age, sex, race, waist circumference, income, T2DM, hypertension, smoking, alcohol intake, caffeine intake, lipids, transferrin saturation	1.17 (0.95-1.43)	8
Lu et al. [39]	2018	China	1,867	54	NA	NA	78.9%	Ultrasound (31.9%)	Urea Breath test (31.5%)	Unadjusted	1.13 (0.92-1.39)	6
Fan et al. [40]	2018	China	21,456	48	23.7	NA	67.1%	Ultrasound (24.3%)	Urea Breath test (50.6%)	Age, sex, BMI, blood pressure, fasting glucose, HbA1c, lipids, uric acid, creatinine	1.0 (0.70-1.30)	10

Yu et al. [41]	2018	China	20,389	48	23.7	4.6%	58.7%	Ultrasound (37.2%)	Urea Breath test (37.2%)	Age, sex, BMI, smoking, white blood cell count, hs-CRP, fasting glucose, triglycerides, GGT	1.14 (1.04-1.38)	10
Mohammadifard et al. [42]	2019	Iran	130	37	NA	NA	47.7%	Ultrasound (50%)	Blood antibodies and fecal antigen test (28.5%)	Unadjusted	1.70 (0.79-3.69)	6
Jiang et al. [43]	2019	China	4,081	45	24.5	8.7%	46.2%	Ultrasound (45.7%)	Urea Breath test (52.4%)	Age, sex, BMI, smoking, education level, hypertension, T2DM, dyslipidemia, metabolic syndrome, transaminases, total bilirubin, urea	1.27 (1.07-1.50)	8
Alvarez et al. [44]	2020	Guatemala	424	55	NA	21.2%	40.3%	HSI >36 (or FLI >60) (62.2%)	Blood antibodies and anti-CagA and VacA IgG antibodies (86.6%)	Unadjusted	1.13 (0.60-2.14)	6
Rahman et al. [45]	2020	Bangladesh	767	40	23.2	12.4%	35.5%	Ultrasound (18.4%)	Blood antibodies (54.5%)	Unadjusted	0.59 (0.40-0.87)	6
Abo-Amer et al. [46]	2020	Egypt	646	37	29.21	NA	50.6%	Fibroscan (+ CAP) or ultrasound (58.8%)	Blood antibodies (83.3%)	Unadjusted	1.46 (0.85-2.44)	6
Wang et al. [47]	2021	China	1,898	37	23.2	NA	64.1%	Ultrasound (26.6%)	Urea Breath test (36.3%)	Unadjusted	1.20 (0.97-1.49)	6
Han et al. [48]	2021	Korea	1,784	55	24.5	NA	83.1%	Fibroscan (+ CAP; steatosis ≥248 dB/m); LSM tertiles: <3.2 kPa, 3.3-4.0 kPa and >4.0 kPa (48.8%)	Blood antibodies (39.7%)	Age, sex, BMI, hypertension, T2DM, fasting glucose, triglycerides, HDL-cholesterol, LSM	0.96 (0.78-1.19)	8
Liu et al. [49]	2021	China	5,665	49	24.32	54.5%	54.5%	Ultrasound (30.2%)	Urea Breath test (37.0%)	Age, BMI, smoking, hypertension, dyslipidemia, uric acid, transaminases, T2DM	Men: 1.05 (0.87- 1.26); Women: 0.90 (0.69-1.18)	8
Yan et al. [50]	2021	China	1,185	42	24.72	NA	30.5%	Ultrasound (44.6%)	Urea Breath test (39.2%)	Age, BMI, carotid plaque(s), uric acid, transaminases, fasting glucose, lipids, blood pressure	1.35 (1.02-1.79)	8
Wernly et al. [51]	2022	Austria	5,338	58	27	16.8%	50.9%	Ultrasound and Fibroscan (in a patient subgroup) (45.5%)	Blood antibodies (19.1%)	Age, sex, BMI, T2DM, LDL-cholesterol	0.96 (0.82-1.13)	7

Wang et al. [52]	2022	China	71,633	45	23.9	5.2%	57.9%	Ultrasound (32.5%)	Urea Breath test (34.5%)	Age, sex, BMI, blood pressure, fasting glucose, HbA1c, lipids, urea	1.02 (0.96-1.07)	8
Chen Y et al. [53]	2023	China	52,032	49	NA	13.8%	62.0%	Ultrasound (20.9%)	Urea Breath test (37.9%)	Unadjusted	1.06 (1.02-1.11)	7
Fialho et al. [54]	2023	USA	91	58	29	39.5%	41.7%	Fibroscan (+ CAP; mild steatosis 220-230 dB/m, moderate steatosis 230-283 dB/m, and severe steatosis >283 dB/m) (73.6%)	Biopsy or fecal antigen test (40.7%)	Sex, hypertension, diabetes, dyslipidemia, or metabolic syndrome	4.27 (1.34-15.9)	6

Abbreviations: BMI, body mass index; CagA, cytotoxin-associated gene A antigen; CAP, controlled attenuation parameter; FLI, fatty liver index; GGT, gamma-glutamyl transferase; hs-CRP, high-sensitivity C-reactive protein; HSI, hepatic steatosis index; LSM, liver stiffness measurement; NAFLD-LFS, NAFLD liver fat score; NA, not available; NOS, Newcastle-Ottawa quality assessment scale; T2DM, type 2 diabetes mellitus; VacA, vacuolating cytotoxin A.

Note: Cut-off values used for CAP and LSM on FibroScan were not clearly specified in all cross-sectional studies.

Table 2. Main baseline characteristics of the eligible retrospective cohort studies (n=4) assessing the association between prior or active *H. pylori* infection and the risk of developing incident MASLD.

Author, Ref	Year	Country	Sample Size (n)	Mean follow-up (years)	Age (years)	BMI (kg/m²)	Percentage of men (%)	MASLD diagnosis (number of incident MASLD cases)	*H. pylori* infection diagnosis at the baseline (% of HP positivity at baseline)	Covariate adjustment	Hazard ratio (95% CI)	NOS scale
Kim TJ et al. [55]	2017	South Korea	17,028 (No information was available in these participants on *H. pylori* eradication status at baseline)	5.1	49	23	51.6%	Ultrasound (3381)	Blood antibodies (58.2%)	Age, sex, BMI, smoking, alcohol consumption, physical activity, education level, year of exam, blood pressure, fasting glucose, lipids, transaminases, hs-CRP, insulin resistance, therapy for hypertension, dyslipidemia, and T2DM	1.16 (1.05-1.30)	8
Abdel-Razik et al. [56]	2018	Egypt	369 (Both patients eradicated and patients uneradicated for *H. pylori* infection were included at baseline)	2.0	NA	NA	NA	HSI >36 (23)	Fecal antigen test (46.3%)	Age, sex, BMI, smoking, socio-economic status, physical activity, education level, fasting glucose, lipids, uric acid, hs-CRP, insulin resistance	1.11 (1.04-1.30)	6
Zhao et al. [57]	2021	China	675 (Patients who had received a complete *H. pylori* eradication were excluded from the study at baseline)	5.0	55	NA	74.9%	Ultrasound (110)	Urea Breath test (30.5%)	Unadjusted	1.19 (0.77-1.83)	6
Kim JW et al. [58]	2022	South Korea	3,780 (Both patients eradicated and patients uneradicated for *H. pylori* infection were included at baseline)	7.9	50	23	56.4%	Ultrasound (1294)	Blood antibodies (19.7%)	Age, sex, BMI, smoking, alcohol consumption, blood pressure, fasting glucose, lipids, transaminases, hs-CRP	1.36 (1.18-1.56)	8

Abbreviations: BMI, body mass index; hs-CRP, high sensitivity C reactive protein; HIS, hepatic steatosis index; NA, not available; NOS, Newcastle-Ottawa quality assessment scale; T2DM, type 2 diabetes mellitus.

Note: no detailed information is available in these cohort studies on the use of *H. pylori* eradication therapies over the follow-up.

FIGURE LEGENDS

Figure 1. Forest plot and pooled estimates of the effect of *H. pylori* infection on the risk of prevalent MASLD in the 24 eligible cross-sectional studies.

Figure 2. Forest plot and pooled estimates of the effect of *H. pylori* infection on the risk of developing incident MASLD in the four eligible retrospective cohort studies.

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