Association Between Nonalcoholic Fatty Liver Disease and Colorectal Tumours in Asymptomatic Adults Undergoing Screening Colonoscopy: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: It is currently uncertain whether non-alcoholic fatty liver disease (NAFLD) is associated with an increased risk of colorectal tumours. We performed a meta-analysis of relevant observational studies to quantify the magnitude of the association between NAFLD and risk of colorectal adenomas and cancer.

Methods: We searched PubMed, Scopus and Web of Science from January 2000 to November 2017 using pre-defined keywords to identify observational studies of asymptomatic adults undergoing screening colonoscopy, in which NAFLD was diagnosed by imaging or histology. Data from selected studies were extracted and meta-analysis was performed using random-effects modelling.

Results: Eleven observational studies (8 cross-sectional and 3 longitudinal) with aggregate data on 91,124 asymptomatic adults of predominantly Asian descent (32.1% with NAFLD) accounting for a total of 14,911 colorectal adenomas and 1,684 cancers were included in the final analysis. NAFLD was associated with an increased risk of prevalent colorectal adenomas (*n*=7 studies using liver imaging techniques; random-effects odds ratio [OR] 1.28, 95%CI 1.11-1.48; I^2=82.9% or *n*=1 study using liver biopsy; random-effects OR 1.61, 95%CI 0.90-2.89) and cancer (*n*=4 studies using liver imaging techniques; random-effects OR 1.56, 95%CI 1.25-1.94; I^2=65.6% or *n*=1 study using liver biopsy; random-effects OR 3.04, 95%CI 1.29-7.18). NAFLD was also associated with an increased risk of incident colorectal adenomas (*n*=3 studies; random-effects hazard ratio [HR] 1.42, 95%CI 1.18-1.72; I^2=0%) and cancer (*n*=1 study; random-effects HR 3.08, 95%CI 1.02-9.03). These risks were independent of age, sex, smoking, body mass index and diabetes (or metabolic syndrome). Sensitivity analyses did not alter these findings. Funnel plot and Egger's test did not reveal significant publication bias.

Conclusions: This meta-analysis of observational studies (involving asymptomatic individuals of predominantly Asian descent undergoing screening colonoscopy) suggests that NAFLD (detected by imaging or biopsy) is independently associated with a moderately increased prevalence and incidence of colorectal adenomas and cancer. However, the observational design of the studies does not allow for proving causality, and the possibility of residual confounding by some unmeasured factors cannot be ruled out. More prospective studies, particularly in European and American individuals,

and mechanistic studies are required to better understand the association between NAFLD and colonic carcinogenesis.

Keywords: NAFLD; colorectal cancer; colorectal tumours; meta-analysis

LIST OF ABBREVIATIONS

BMI, body mass index
CI, confidence interval
HCC, hepatocellular carcinoma
HR, hazard ratio
NAFLD, non-alcoholic fatty liver disease
NASH, non-alcoholic steatohepatitis
NFS, NAFLD fibrosis score
NOS, Newcastle-Ottawa Quality Assessment Scale
OR, odds ratio
PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of progressive liver conditions, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis and even hepatocellular carcinoma (HCC). NAFLD has become one of the most common chronic liver diseases worldwide, and its prevalence is projected to rise dramatically over the next decade [1-3]. Strong evidence supports the view that the clinical and economic burden of NAFLD is not only restricted to severe liver-related complications, but also includes major extra-hepatic diseases that exert considerable effects on health-care expenditure [4-7]. Indeed, it is known that cardiovascular disease is the leading cause of mortality among patients with NAFLD, followed by extra-hepatic cancers and liver-related complications [4-6].

In this regard, the relationship between NAFLD and risk of colorectal tumours has recently gained considerable scientific interest [6]. Colorectal cancer is the third most commonly diagnosed malignancy and the fourth leading cause of cancer-related deaths in the world, and its burden is expected to increase by nearly 60% to more than 2.2 million new cases and 1.1 million cancer deaths by 2030 [8]. The geographic distribution of colorectal cancer burden varies widely, with more than two-thirds of all new cases and about 60% of all cancer-related deaths occurring in high-income countries [8]. Therefore, the high mortality rates and the increasing health-care costs associated with colorectal tumours have led investigators to identify novel and potentially modifiable risk factors.

NAFLD and colorectal tumours share many metabolic risk factors (mainly type 2 diabetes and obesity) and proinflammatory and profibrotic molecular pathways [9,10]. To our knowledge, there are currently only two small meta-analyses of observational studies (published in 2014 and 2015 respectively) suggesting that NAFLD may be associated with an increased risk of colorectal tumours in asymptomatic individuals undergoing screening colonoscopy [11,12]. However, we believe that the results of these two small meta-analyses should be interpreted cautiously, because they have

included a relatively small number of studies (*i.e.*, five or seven observational studies, involving a total of up to 11,900 subjects with a very low number of colorectal cancers), the majority of which were cross-sectional and where, in some cases, the diagnosis of NAFLD was only based on surrogate diagnostic markers, such as serum liver enzymes [11,12]. Thus, whether NAFLD is a possible new risk factor for colorectal tumours remains uncertain.

As will be discussed in detail later, a number of large observational studies involving asymptomatic adults undergoing screening colonoscopy have been published after the publication of these two aforementioned meta-analyses. In all these published studies the diagnosis of NAFLD was mainly based on ultrasonography, which is the recommended first-line non-invasive method for detecting NAFLD in clinical practice [1,2].

Thus, we included these novel observational studies in an updated evaluation, and herein report the results of our comprehensive systematic review and meta-analysis of cross-sectional and longitudinal studies that examined the association between NAFLD (assessed by imaging or histology) and the risk of colorectal tumours. Our aim was to precisely gauge the magnitude of the association between NAFLD and risk of both colorectal adenomas and cancer in asymptomatic individuals undergoing screening colonoscopy. We also tested whether the severity of NAFLD was associated with an even greater risk of these tumours. Given the clinical and cost-effectiveness of colorectal cancer screening programmes [13,14], clarification of the magnitude of risk of colorectal tumours associated with the different stages of liver disease within the spectrum of NAFLD would have important clinical implications for the prevention, diagnosis and treatment of colorectal tumours.

2. MATERIALS AND METHODS

Registration of review protocol

The protocol for this systematic review and meta-analysis was registered in advance with PROSPERO (International Prospective Register of Systematic Reviews, *no*. CRD42017081517).

Data sources and searches

We conducted a literature search from January 2000 to 20 November 2017 of PubMed, Scopus and Web of Science for observational studies examining the association between NAFLD and risk of colorectal adenomas and cancer. Search free text terms were “non-alcoholic fatty liver disease” (OR “fatty liver” OR “nonalcoholic steatohepatitis) AND “colorectal neoplasms” OR “colorectal cancer” OR “colorectal adenomas”. Searches were restricted to human studies. No language restrictions were imposed. Additionally, we reviewed references from relevant original papers and review articles to identify further eligible studies. We performed a systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Because the included studies were observational in design, we followed the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines for the meta-analysis of these studies [15].

Study selection

We included only large (*n* ≥300) cross-sectional or longitudinal studies of asymptomatic adults (>18 years old) undergoing screening colonoscopy, and where the diagnosis of NAFLD was based on either liver biopsy or imaging techniques, in the absence of excessive alcohol consumption and other competing causes of chronic liver disease. Study participants were of either sex with no restrictions in terms of race, ethnicity or comorbidities.

Criteria for exclusion of selected studies from our meta-analysis were as follows: 1) congress abstracts, case reports, reviews, commentaries, and editorials; 2) studies where NAFLD diagnosis was based exclusively on serum liver enzymes; 3) studies which did not exclude individuals with excessive alcohol consumption and other known

causes (*e.g.*, viral, drugs) of chronic liver diseases; 4) studies in which the outcome measure was not diagnosed by a screening colonoscopy; 5) studies performed in cirrhotic patients evaluated for liver transplantation who underwent screening colonoscopy; 6) cross-sectional studies with a case-control design; 7) studies which did not specifically report any odds (OR) or hazard ratio (HR) and 95% confidence intervals for the outcome measure; and 8) studies conducted in paediatric populations.

Two investigators (AM and GT) independently examined all titles and abstracts, and obtained full texts of potentially relevant papers. Working independently and in duplicate, we read the papers and determined whether they met inclusion criteria. Discrepancies were resolved by consensus, referring back to the original article, in consultation with a third author (MD).

Data extraction and quality assessment

For all studies, we extracted information on study design, study size, year of publication, study country, participant characteristics, methods used for NAFLD diagnosis, follow-up duration, outcome of interest (colorectal adenomas or cancer) and covariates adjusted in multivariate regression analyses. In the case of multiple publications, we included the most up-to-date or comprehensive information. We also contacted three corresponding authors of the eligible studies in order to obtain additional information, but only one responded providing extra-information to us (as reported in the Acknowledgments section).

Two authors (AM and GT) assessed the risk of bias independently. Any discrepancies were addressed by a revaluation of original articles by a third author (MD). Quality assessment was performed according to the Newcastle-Ottawa Quality Assessment Scale (NOS), which is a validated scale for non-randomized studies in meta-analyses [16]. We used a NOS scale adapted for the cross-sectional studies [16]. 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 four stars for

selection (or five stars in the case of cross-sectional studies), two stars for comparability, and three stars for outcome/exposure. We judged studies that received a score of at least eight stars to be at low risk of bias (*i.e.*, thus reflecting the highest quality).

Data synthesis and analysis

The outcome measure was the presence (or the occurrence over the follow-up) of colorectal adenomas or cancer on screening colonoscopy among asymptomatic adults with NAFLD in comparison with the risk of colorectal adenomas or cancer among those without NAFLD. The ORs (for cross-sectional studies) or HRs (for longitudinal studies) and 95% confidence intervals were considered as the effect size for all the eligible studies. When studies had several adjustment models, we extracted those that reflected the maximum extent of adjustment for potentially confounding variables.

The adjusted OR/HR values of all studies were pooled, and an overall estimate of effect size was calculated using a random-effects model, as this methodology takes into account any differences between studies even if there is no statistically significant heterogeneity.

Visual inspection of the forest plots was used to investigate the possibility of statistical heterogeneity. The statistical heterogeneity among studies was assessed by the I^2 statistic, which provides an estimate of the percentage of variability across studies that is due to heterogeneity rather than chance alone. According to Higgins and Thompson [17], a rough guide to interpretation is as follows: I^2 values of approximately 25% represent low heterogeneity; approximately 50% represent medium heterogeneity; and approximately 75% represent high heterogeneity.

The possibility of publication bias was evaluated using the funnel plot and the Egger's regression asymmetry test [18].

To explore the possible sources of heterogeneity among studies and to test the robustness of the associations, we conducted sensitivity/subgroup analyses by study design (cross-sectional *vs*. longitudinal), study country, diagnostic methods of NAFLD (imaging *vs*. biopsy), severity of NAFLD (based on biopsy, ultrasonographic steatosis scores or non-invasive fibrosis markers, such as NAFLD fibrosis score [NFS]), levels of body mass index (BMI ≥24 *vs*. <24 kg/m^2, based on the mean BMI value of the pooled eligible studies), and quality of studies (based on the NOS scale or whether the studies had full adjustment for known risk factors [*i.e*., those studies adjusting at least for age, sex, BMI, smoking, diabetes or metabolic syndrome]). We also performed sensitivity analyses stratifying the outcome measure by type of histologic colorectal lesion (adenoma *vs*. cancer) or, if available, by presence of multiple adenomas (≥3 *vs*. <3). Additional sensitivity analyses were also performed by omitting one study at a time and calculating a pooled estimate for the remainder of the studies to evaluate whether the results were significantly affected by a single study.

All statistical tests were two-sided and used a significance level of $p<0.05$. We used STATA® 14.2 (StataCorp, College Station, Texas) for all statistical analyses.

3. RESULTS

Literature search and study characteristics

Figure 1 shows the results of the literature research and study selection. After excluding duplicates, based on the titles and abstracts of 341 citations (in accordance with the aforementioned exclusion criteria of the meta-analysis), we initially identified 19 potentially relevant studies from PubMed, Scopus and Web of Science prior to 20 November 2017. After evaluating the full text of these 19 publications, we further excluded eight studies as specified in the PRISMA flow diagram. In total, eleven observational studies (8 cross-sectional and 3 longitudinal) were eligible for inclusion in the meta-analysis and were assessed for quality [19-29]. In **supplementary Table 1**

are specified the syntax used and the records identified through database searching. In the **supplementary Table 2,** we summarized the characteristics of the eight excluded studies (at the stage of eligibility according to the PRISMA flow diagram) with the exact reason(s) for exclusion.

The main characteristics of the 11 included studies are shown in **Table 1**. These studies recruited adult individuals from approximately general populations, who underwent a screening colonoscopy, and where the diagnosis of NAFLD was based on either liver biopsy (*n*=1 study) or imaging techniques (ultrasonography, *n*=10 studies; computed tomography, *n*=1 study; magnetic resonance spectroscopy, *n*=1 study), in the absence of excessive alcohol consumption and other competing causes of chronic liver disease. Most of these studies were carried out in Asia (South Korea, China and Taiwan); only a study was carried out in Europe (Austria). Most of these studies included middle-aged, non-obese individuals (mean age: 52 years; mean BMI: 24 kg/m^2), predominantly of male sex. Eight studies had a cross-sectional design, whereas three studies had a longitudinal (retrospective) cohort design.

Overall, in the eleven studies included in the meta-analysis there were 91,124 asymptomatic individuals (32.1% with NAFLD; *n*=29,319) with a total of 14,911 colorectal adenomas and 1,684 cancers or advanced neoplasms. In particular, in the eight cross-sectional studies there were 83,062 individuals (33% with NAFLD) with a total of 14,396 adenomas and 1,656 cancers [19-26]. The overall cumulative prevalence of colorectal adenomas was 20.4% (95%CI 19.9-20.9) in patients with NAFLD and 15.8% (95%CI 15.5-16.1) in those without NAFLD, whereas the prevalence of colorectal cancer was 2.4% (95%CI 2.2-2.6) and 1.97% (95%CI 1.9-2.0), respectively.

In the three longitudinal studies there were 8,062 Asian individuals (24% with NAFLD) with a total of 515 incident colorectal adenomas and 28 incident cancers or advanced neoplasms occurring over a median follow-up of 4.5 years (interquartile range: 3.5-5.0

years) [27-29]. The overall cumulative incidence rate of colorectal adenomas was 16.1% (95%CI 15.9-16.2) in patients with NAFLD and 3.4% (95%CI 2.9-3.8) in those without NAFLD, whereas the incidence rate of colorectal cancer was 1.6% (95%CI 0.70-2.41) and 0.05% (95%CI 0.01-0.10), respectively.

Of the eight cross-sectional studies (**supplementary Table 3**), seven studies received at least eight stars on the NOS (indicating that those studies had a low risk of bias), and only one study received six stars (*i.e*., being at high risk of bias). Conversely, all longitudinal studies received six stars on the NOS.

Risk of prevalent colorectal tumours

The distribution of studies by estimate of the association between NAFLD and risk of prevalent colorectal tumours is plotted in **Figure 2**.

NAFLD was moderately associated with an increased risk of prevalent colorectal tumours (overall random-effects OR 1.40, 95%CI 1.24-1.57; I^2=78.8%). As we have always used the fully adjusted OR estimates for each eligible study (as specified in **Table 1**), this random-effects OR was independent of age, sex, BMI, smoking, hypertension, diabetes or metabolic syndrome.

Most importantly, as also shown in **Figure 2**, the significant association between NAFLD and risk of prevalent colorectal tumours was consistent both for adenomas and for cancer, irrespective of the methods used for diagnosing NAFLD (ultrasonography or biopsy/^{1}H-MRS).

Risk of incident colorectal tumours

The distribution of studies by estimate of the association between NAFLD and risk of incident colorectal tumours is plotted in **Figure 3**. Three longitudinal Asian studies provided data suitable for the pooled primary analysis [27-29].

NAFLD was moderately associated with an increased risk of incident colorectal tumours (overall random-effects HR 1.47, 95%CI 1.20-1.81; I^2=11.5%). Notably, since we have always used the fully adjusted HR estimates for each eligible study, this overall random-effects HR was independent of common metabolic risk factors.

As also shown in **Figure 3**, the significant association between NAFLD and risk of incident colorectal tumours was consistent both for adenomas (*n*=3 studies; random-effects HR 1.42, 95%CI 1.18-1.72; I^2=0%) and for cancer (*n*=1 study; random-effects HR 3.08, 95%CI 1.02-9.03).

Subgroup and sensitivity analyses and meta-regression

To explore possible sources of heterogeneity across studies, we carried out several sensitivity analyses (**Table 2**).

Limiting the analysis to “high-quality” cross-sectional studies with NOS >8 stars or those with full adjustment for covariates provided overall estimates consistent with the pooled primary analysis for both colorectal adenomas and cancer. This analysis was not performed in longitudinal studies, because all these studies received six stars on the NOS.

When the comparison was stratified by either BMI category or study country (although the vast majority of the eligible studies included Asian people), the significant association between NAFLD and risk of colorectal adenomas or cancer was consistent in both cross-sectional and longitudinal studies.

As also shown in **Table 2** (last rows), two Asian studies also reported a significant, graded relationship between NAFLD and risk of prevalent or incident multiple (≥3) colonic adenomas. However, more research is needed to confirm this finding.

We also tested for the possibility of excessive influence of individual studies using an influence test that eliminated each of the included studies one at a time. Eliminating each of the eligible studies from the analysis had no significant effect on the overall risk of colorectal adenomas or cancer (data not shown).

In **supplementary Figure S1** we reported the results of univariate meta-regression analyses showing the lack of any significant association of age, sex, BMI, waist circumference (available only for three studies), and NOS scale with the risk of prevalent colorectal tumours in the eligible cross-sectional studies.

As shown in **supplementary Figure 2,** the Egger's regression test did not show statistically significant asymmetry of the funnel plot (p=0.08), thus suggesting that publication bias was unlikely, although it should be noted that the numbers of included studies (n=11) was relatively small.

NAFLD severity and risk of prevalent colorectal tumours

The distribution of studies by estimate of the association between more 'severe' NAFLD and risk of prevalent colorectal tumours is plotted in **Figure 4**. Three cross-sectional studies (involving a total of 71,140 individuals with 11,191 colorectal adenomas and 1,077 cancers; 36.8% had NAFLD) provided data suitable for the pooled primary analysis [21,23,24]. In these studies the severity of NAFLD was defined by biopsy (n=1 study including 135 Chinese individuals), by ultrasonographic steatosis scores (n=1 study including 44,220 South Korean individuals) or by the use of non-invasive markers of fibrosis (n=1 study including 26,540 South Korean individuals). No longitudinal studies were available for this analysis.

Overall, the severity of NAFLD was associated with a ~twofold increased risk of prevalent colorectal tumours (random-effects HR 2.12, 95%CI 1.56-2.88; I^2=68.1%).

With regards to the methods used for staging NAFLD severity, the risk of colorectal adenomas and cancer appeared to be slightly greater in the single study by Wong *et al*. [21], who used liver biopsy for staging NAFLD severity (NASH *vs*. simple steatosis), compared to studies using either ultrasonographic steatosis scores or non-invasive markers of fibrosis.

However, it should be noted that neither cross-sectional nor longitudinal studies involving American or European individuals were available for this latter analysis, thus limiting the generalizability of these findings to other ethnicities.

4. DISCUSSION

Our meta-analysis exploring the association between NAFLD and risk of colorectal tumours is the largest and most comprehensive assessment to date. Meta-analysis of data from the eight cross-sectional studies has shown that imaging-based or liver-biopsy proven NAFLD was associated with a moderately increased risk of prevalent colorectal adenomas and cancer. These risks seemed to increase further with greater severity of NAFLD (in studies using liver biopsy, ultrasonographic steatosis scores or NAFLD fibrosis score) and, importantly, remained significant in those studies where analysis was fully adjusted for age, sex, smoking, BMI, diabetes or other metabolic risk factors. More interestingly, meta-analysis of data from the three longitudinal Asian studies has shown that imaging-diagnosed NAFLD was associated with a 42% increased long-term risk of incident colorectal adenomas and with a three-fold increased long-term risk of incident colorectal cancer, even after adjusting for age, sex, smoking and metabolic risk factors.

The issue of whether the increased risk for colorectal tumours is restricted to patients with more severe forms of NAFLD (*i.e*., NASH and/or advanced fibrosis, estimated to represent up to 10-15% of the overall NAFLD population) [1,3] or applies to all patients

with NAFLD, is particularly relevant in view of the disease burden imposed by NAFLD on healthcare systems. The results of our meta-analysis suggest that it is advanced NAFLD that might confer a greater risk of colorectal tumours. Our findings have been confirmed by a recent cohort study of 25,947 South Korean individuals showing that ultrasound-diagnosed NAFLD was independently associated with an increased risk of developing HCC and colorectal cancer especially in men over a median follow-up of 7.5 years, and that this risk was progressive with advancing hepatic fibrosis scores [30]. Moreover, these findings are also consistent with the conclusions of recent meta-analyses supporting a significant, graded association between the severity of NAFLD and risk of developing incident cardiovascular events [31] or other extrahepatic diseases [32-34]. However, this question remains largely unsolved, and further prospective studies are needed to definitely prove whether the severity of NAFLD affects the risk of developing colorectal tumours.

We believe that the findings of our meta-analysis have potential clinical implications, suggesting that a diagnosis of NAFLD could identify a subset of individuals, who are at higher risk of having colorectal adenomas or cancer, and who could need more careful surveillance. Most of the eligible cohort studies did not show a clear sex-difference in the NAFLD-related risk of colorectal tumours, whereas a few cohort studies included in the meta-analysis suggested that NAFLD may be associated with a higher risk of multiple (≥3) colonic adenomas and more colon cancer located in right colon. However, further larger studies are needed to confirm these findings. In addition, it is important to underline that no prospective cancer screening trials in patients with NAFLD have been performed to support screening beyond the current cancer screening guidelines. Therefore, although a diagnosis of NAFLD is not currently sufficient to recommend screening colonoscopy [35], evaluation of colonic symptoms and ensuring patients are enrolled in colorectal screening programmes as per recommendations for the general population is recommended. Recently, Wong *et al*. evaluating the cost-effectiveness of different colorectal cancer screening strategies highlighted the higher cost-effectiveness of colonoscopy screening performed once every 10 years starting at age 50 for patients with NAFLD [36]. However, further

evidence is needed to clarify the risk in patients aged 40–50 years, who are not currently within routine cancer screening guidelines.

From a pathophysiological perspective, it is currently uncertain whether NAFLD is associated with an increased risk of colorectal tumours simply as a consequence of the shared metabolic risk factors, or whether NAFLD itself may contribute to the development of colorectal tumours, irrespective of shared metabolic risk factors. The close interconnections between NAFLD, abdominal obesity, diabetes and insulin resistance make it extremely difficult to distinguish the precise causal relationships underlying the increased risk of colorectal tumours in patients with NAFLD. It is becoming increasingly clear that the liver and gut share a number of pathophysiological pathways that are intrinsically linked to each other [37-39]. Strong evidence indicates that the risk of colorectal tumours is increased in individuals with abdominal obesity, diabetes or metabolic syndrome [40-42]. Recent research has characterized important pathways that might link metabolism, low-grade inflammation and cancer development [9,10,43]. Mediators derived mainly from the adipose tissue, such as adiponectin and other adipokines, could be attractive candidates as the missing link between abdominal obesity, NAFLD/NASH and cancer development [44-46]. Experimentally, adiponectin has beneficial effects on colonic carcinogenesis in mice [47]. On this background of evidence, it is reasonable to assume that the biologic mechanisms potentially responsible for accelerated colonic carcinogenesis in NAFLD probably have their origin in the expanded and inflamed visceral adipose tissue, with the liver being both the target of the resulting systemic abnormalities and a source of both altered bile acid pool and multiple proinflammatory, prooxidant and profibrogenic molecules that might contribute to the development of colorectal tumours and other extra-hepatic diseases [5,6,9,10,39,48,49]. However, a greater understanding of the key pathways linking metabolism, low-grade systemic inflammation and colorectal cancer development is awaited.

Our meta-analysis has some important limitations (strictly inherent to the design of the included studies) that should be mentioned. First, the observational design of the

eligible (cross-sectional and longitudinal) studies does not allow establishing temporal or causal relationships between NAFLD and risk of colorectal tumours. In addition, since both conditions are asymptomatic and detected on screening, causality is difficult to establish and findings could be explained by healthy user bias. Second, although almost all studies included in the meta-analysis have adjusted the results for age, sex, smoking, BMI and pre-existing diabetes (or metabolic syndrome), the possibility of residual confounding by some unmeasured factors cannot be ruled out. In fact, the majority of the eligible studies reported incomplete adjustments for known risk factors and potential confounding variables (*i.e*., family history of colorectal cancer, dietary factors, physical activity, waist circumference, and circulating levels of certain adipokines, such as adiponectin); in particular, we consider that the lack of any statistical adjustment in all studies included in the meta-analysis for waist circumference or other measures of intra-abdominal fat accumulation represents one of the major weaknesses of these studies. Only three cross-sectional studies included in the meta-analysis reported data on waist circumference [19-21], but did not adjust the results also for this covariate. In these three available studies, we performed a meta-regression analysis that did not reveal a significant association between waist circumference and risk of prevalent colorectal adenomas (supplementary Figure S1). Other studies included in the meta-analysis adjusted the results for metabolic syndrome, but in all cases the presence of abdominal obesity was defined as BMI ≥25 kg/m^2 following the China Diabetes Federation metabolic syndrome criteria [25,26,28]. That said, we believe that it is essential that measurement of waist circumference or other measures of intra-abdominal fat accumulation is always performed in all future studies to better define the independent contribution of NAFLD on the risk of developing colorectal tumours. Third, although we used a random-effects model, the interpretation of some results of this meta-analysis requires some caution, given the high heterogeneity observed especially in the pooled primary analysis of cross-sectional studies (I^2=78.8%). It is plausible to assume that the high heterogeneity of the cross-sectional studies likely reflects differences in the characteristics of study populations, in the study country as well as in the methods used for diagnosing NAFLD, and in the type of histologic colorectal lesions. It is also possible that there is variable baseline risk for the development of colorectal tumours between studies. We

systematically explored and identified possible sources of statistical heterogeneity using stratified analyses, meta-regression and sensitivity analyses. Although we found a significant heterogeneity between the cross-sectional studies when investigating associations in the pooled primary analysis, it is noteworthy that there was a lower heterogeneity between these studies when we restricted the analyses to the "high-quality" studies or to those examining the association between NAFLD severity and risk of colorectal cancer. However, we believe that more detailed analyses of the causes of heterogeneity will require collaborative pooling of individual participant data from large prospective studies as these become available over time. Fourth, another potential limitation of this meta-analysis is that most of the studies used liver ultrasonography which is the recommended first-line imaging method for detecting NAFLD in clinical practice [50], whereas only one study used liver biopsy which is the reference standard for diagnosing and staging NAFLD [1,2,50]. Fifth, most of the eligible studies, except one [20], originate from Asian countries, where large populations undergo regular health check-up programs, including liver ultrasonography. Although the only cross-sectional study of Austrian individuals included in this meta-analysis showed a positive, independent association between NAFLD and risk of colorectal adenomas [20], however, the generalizability of these findings to European and US populations remains uncertain. Also, the colorectal cancer screening programs in the US/Europe might not be easily compared with the health check-up programs in Asian countries, and thus any recommendations could be misleading at this time. The overall detection rates of colorectal adenomas and cancer across all eligible studies were respectively 17.3% and 2.2%, which are lower compared with those detected in cancer screening programs in the US and Europe (estimated to be around 25-30% and 8-10%, respectively) [8,51,52]. Therefore, it cannot be excluded that the observed effect of NAFLD on risk of colorectal tumours could be lost in populations with a higher 'a priori' risk of colorectal cancer, namely in the US and Europe. As Asian and non-Asian populations have different lifestyle habits, body fat distribution and cultural/genetic backgrounds that might have profound effects on colorectal cancer development, further larger observational cohort studies should be conducted in non-Asian populations. Finally, although a selective reporting bias of eligible studies could be not definitely excluded, we also searched for 'grey' literature

in Scopus and Web of Science databases and made every effort to rule out very low-quality studies by using stringent inclusion criteria. We believe that our comprehensive search has made it unlikely that any published reports were missed and visual inspection of funnel plots and formal tests demonstrated no statistical evidence of publication bias (although the interpretation of the Egger's test should be viewed cautiously because the number of studies included was relatively low).

Notwithstanding these limitations, our meta-analysis has also important strengths. As previously discussed, the present meta-analysis provides the most comprehensive assessment to date on the association between NAFLD and risk of colorectal adenomas and cancer. These results, obtained by analyzing nearly 15,000 colorectal adenomas and nearly 1,700 cancers among 91,124 asymptomatic adults undergoing screening colonoscopy suggest that the prevalence and incidence of colorectal adenomas and cancer in individuals with NAFLD are moderately higher than those observed in individuals without NAFLD, especially in Asian individuals. Finally, we employed standardized risk estimates from all eligible studies to allow a consistent combination of estimates across studies. The large number of cases of colorectal adenomas and cancer has provided high statistical power to quantitatively assess the association between NAFLD and risk of colorectal adenomas and cancer.

In conclusion, the findings of this comprehensive meta-analysis of observational studies (involving asymptomatic adults of predominantly Asian descent who underwent screening colonoscopy) suggest that NAFLD is associated with a moderately increased prevalence and incidence of colorectal adenomas and cancer. However, it should be noted that the observational design of the eligible studies does not allow for proving causality, and further studies are needed to draw firm conclusions about any independent hepatic contribution to the increased risk of colorectal tumours observed among patients with NAFLD. Moreover, mechanistic studies are also needed to elucidate the biological mechanisms underlying the association between NAFLD and risk of colorectal tumours.

Conflicts of Interest: All authors declare no conflicts of interest.

Authors Contributions: study concept and design: GT; acquisition of data: AM, MD, GT; statistical analysis of data: AM, GT; analysis and interpretation of data: AM, GT; drafting of the manuscript: GT; critical revision of the manuscript for important intellectual content: CDB, AL, GZ, EB. All authors have approved the submitted manuscript.

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FIGURE LEGENDS

Figure 1. The PRISMA flow diagram of the meta-analysis.

Figure 2. Forest plot and pooled estimates of the effect of NAFLD on the risk of prevalent colorectal tumours in eight eligible cross-sectional studies of asymptomatic adults undergoing screening colonoscopy, stratified by colorectal adenomas and cancer, and methods used for diagnosing NAFLD.

Figure 3. Forest plot and pooled estimates of the effect of NAFLD on the risk of incident colorectal tumours in three eligible longitudinal studies of asymptomatic adults undergoing screening colonoscopy (followed for a median of 4.5 years), stratified by colorectal adenomas and cancer.

Figure 4. Forest plot and pooled estimates of the effect of the severity of NAFLD (defined by liver biopsy, ultrasonographic steatosis scores or NAFLD fibrosis score) on the risk of prevalent colorectal tumours in three eligible cross-sectional studies of asymptomatic adult individuals undergoing screening colonoscopy, stratified by colorectal adenomas and cancer, and methods used for staging NAFLD severity.

Figure S1. Univariate meta-regression analysis. A meta-analysis of the association of age, body mass index (BMI), waist circumference (available only for three studies), Newcastle-Ottawa Quality Assessment Scale (NOS) or sex (male percentage) with the risk of either prevalent colorectal adenomas (panels from A to E) or prevalent colorectal cancer (panels from F to I). Only cross-sectional studies were included in these analyses.

Figure S2. Funnel plot of standard error by log-odds ratio for the risk of colorectal adenomas (cross-sectional and longitudinal studies have been marked differently in the figure).