Use of proton pump inhibitors (PPIs) could predispose individuals to small intestinal bacterial overgrowth (SIBO) by altering the intraluminal environment and bacterial flora. There is controversy regarding the risk of SIBO among PPI users because of conflicting results from prior studies. A systematic review and meta-analysis were performed to evaluate the association between PPI use and SIBO, using objective clinical outcome measures.

Clinical studies comparing SIBO risk among adult users of PPIs vs nonusers were identified in MEDLINE/PubMed, EMBASE, the Cochrane Central Register of Controlled Trials, and the National Institutes of Health Clinical Trials databases through July 2012. Two reviewers independently extracted data on study characteristics and outcomes. The primary metameasure was the odds ratio (OR) of SIBO among PPI users vs nonusers. Subgroup analyses were performed to examine the influence of study characteristics, such as SIBO diagnostic modality, on study outcome.

Eleven studies (n = 3134) met inclusion criteria. The pooled OR of SIBO in PPI users vs nonusers was 2.282 (95% confidence interval [CI], 1.238–4.205). No significant single large study or temporal effect was seen. Subgroup analysis revealed an association between SIBO and PPI use in studies that used duodenal or jejunal aspirate cultures to diagnose SIBO (OR, 7.587; 95% CI, 1.805–31.894), but no relationship was found between SIBO and PPI use in studies that used the glucose hydrogen breath test (OR, 1.93; 95% CI, 0.69–5.42). Funnel plot analysis identified 4 outlying studies, indicating the possible presence of publication bias.

PPI use statistically was associated with SIBO risk, but only when the diagnosis was made by a highly accurate test (duodenal or jejunal aspirate culture). Differences in study results could arise from the use of different tests to diagnose SIBO.

Keywords: Hypochlorhydria; Drug; Small Bowel Flora; Reflux; Side Effect.
bacterial adhesion. Chronic acid suppression and the resultant hypochlorhydria associated with PPI use have been hypothesized to alter the intraluminal environment to promote growth of the bacterial flora in the small intestine. Several reports have linked hypochlorhydria to increased gastric and duodenal bacterial colonization, which have been shown to predispose patients to SIBO development. Gastric hypochlorhydria has also been implicated as a contributing factor in the risk of developing SIBO in elderly and human immunodeficiency virus patients.

In 1994, 2 initial reports were published examining the possible association between PPI use and risk of SIBO. The 2 studies used varying methodologies and design, including different diagnostic testing and criteria for SIBO. Conflicting outcomes regarding the risk of SIBO associated with PPI use resulted from these 2 initial reports. Since then, a number of cohort and case-controlled studies have been implemented in an attempt to clarify the magnitude and directionality of the risk, with varying results. The relationship between PPI use and SIBO, therefore, remains controversial and confusing for both patients and clinicians. With the widespread use and availability of over-the-counter PPI, a better assessment of its side-effect profile and link to other conditions, such as SIBO, is essential.

A previously published review discussed the possible role of PPIs as confounders in the association between SIBO and irritable bowel syndrome. The objective of this meta-analysis was to review available published peer-reviewed evidence to evaluate objectively the relationship between PPI use and diagnosis of SIBO.

Methods

Data Sources and Searches

An electronic search was performed using MEDLINE/PubMed (1950 to July 2012), EMBASE (1980 to July 2012), the Cochrane Central Register of Controlled Trials, and the US National Institutes of Health Clinical Trials databases for case-control studies, cohort studies, or clinical trials of PPI use and SIBO published through July 2012. The following search terms were used as both keywords and medical subject heading terms, where applicable: proton pump inhibitor, PPI, omeprazole, lansoprazole, pantoprazole, esomeprazole, rabeprazole, dexlansoprazole, malabsorption syndromes, bacterial overgrowth. No language restrictions were applied.

Study Selection

The authors independently reviewed the results of the search strategy. The titles, abstracts, and keywords of identified articles were screened for relevance and the reference lists of these articles were examined individually for additional pertinent studies. To be included in this meta-analysis, studies were required: (1) to be randomized controlled trials, case-control studies, or cohort studies of SIBO risk in general adult (age ≥18 y) PPI users vs nonusers; (2) to diagnose SIBO by one of the currently available clinical tests, namely small-bowel aspirate culture or breath testing; and (3) to include a non-PPI control group. To minimize heterogeneity, studies that were published in abstract form were not included. Studies that focused exclusively on a specific subpopulation of patients at increased risk for SIBO, such as patients with scleroderma or postabdominal surgery, were excluded because some of these conditions may be effect modifiers, thus skewing the association between PPI use and SIBO.

Data Extraction and Quality Assessment

Two independent reviewers extracted data and assessed the quality of the selected studies with no discrepancies. The following clinical data were identified a priori and extracted from each study, where possible: total number of subjects with and without SIBO off PPI; total number of subjects with and without SIBO on PPI; adjusted odds ratio (OR) of SIBO in PPI users vs nonusers; geographic region of study; testing modality to diagnose SIBO; cut-off values for hydrogen breath testing; number of subjects; mean age of subjects; and type, dose, and duration of PPI exposure. Formal methodologic quality was assessed using the Newcastle-Ottawa Quality Assessment Scale for nonrandomized studies. All included studies were rated 6 to 8 of a maximum score of 9, and were of sufficient quality for inclusion in the meta-analysis.

Data Synthesis and Statistical Analysis

The primary metameter for this study was the OR for SIBO among PPI users vs nonusers. To quantify the variation across studies caused by heterogeneity, the Cochran Q and I² statistics were calculated, with a P value less than .05 for the Q statistic or I² greater than 20% considered significant for heterogeneity. Potential sources for clinical heterogeneity among studies were specified a priori, including testing modality and study design, for which subgroup analyses were performed. These subgroups were analyzed further by stratification to assess for possible interactions between these factors. Because of anticipated variability in patient population and study design, we used the DerSimonian–Laird random effects model for pooled analysis with significance accepted at a P value less than .05. A one-study–removed analysis was performed on the pooled data to examine the effect of single large studies. A cumulative analysis was also conducted to explore time trends by publication year. The possibility of publication bias was assessed with a funnel plot and adjusted with Duval and Tweedie trim-and-fill method. All analyses were conducted using Comprehensive Meta-Analysis, version 2 (Biostat, Englewood, NJ). The meta-analysis was performed in accordance with the Meta-analysis Of Observational Studies in Epidemiology guidelines.

Results

Study Selection

The search strategy yielded 305 articles, of which 12 full-text studies were retrieved. The excluded 293 articles included duplicate citations, non-peer-reviewed abstracts, animal studies, case reports or series, editorials, letters, reviews, and practice guidelines. Of the 12 studies identified, 1 was excluded for focusing on a specific subpopulation of patients with increased baseline risk of SIBO development; however, the study was small and had only 10 subjects, so its inclusion would have had only a minimal impact on the overall pooled results. The resulting 11 publications represented 11 discrete study cohorts that were included in the meta-analysis. Figure 1 shows a flow chart documenting the study selection process.
In all, 11 study cohorts were identified that reported SIBO risk in PPI users vs nonusers (n = 3134) (Table 1). Nine studies were prospective cohort studies,8,13–19,21 and 2 were retrospective reviews.20,22 Seven studies13,16–20,22 used a separate cohort of PPI nonusers for comparison, whereas the remaining 4 studies8,14,15,21 compared outcomes in the same cohort before and after PPI exposure. Five studies used duodenal/jejunal aspirate culture obtained via small-bowel intubation for the diagnosis of SIBO,8,13,15,17,20 whereas the remaining 6 studies used a variety of breath testing, including the 14C-glycocholate breath test,14 glucose hydrogen breath test (GHBT),17,19,21,22 or lactulose hydrogen breath test.18 One study7 used the lactulose hydrogen breath test as an additional confirmatory test in a minority of patients (n = 6) after diagnosis of SIBO by duodenal/jejunal aspirate, and a second study15 used 13C-D-xylose breath testing for diagnosis of SIBO in addition to jejunal aspirate; these data were omitted from analysis to prevent double counting of subjects. Another study22 compared test results of 4 different cut-off thresholds for SIBO diagnosis in GHBT. Data from the cut-off threshold of either H2 greater than 20 or CH4 greater than 15 was used for this meta-analysis because this reflects the most commonly accepted practice in clinical SIBO testing currently. The mean age of subjects across all studies was 52.4, with women comprising 64.3% of the total study population. All studies had a Newcastle–Ottawa score between 6 and 8, and were of acceptable methodologic quality for inclusion in the meta-analysis.

**Meta-analysis**

Across all studies, a statistically significant level of heterogeneity was observed (Q test, 61.2; P < .001; I², 83.7), which was likely attributable to the various testing modalities and cut-off ranges used in the diagnosis of SIBO. In the analysis of the primary metamester, 6 of the 11 included studies showed an increased risk of SIBO with PPI use, as calculated from the extracted numeric data. The overall meta-analysis revealed a statistically significant increase in risk of SIBO after applying...
the random-effects model to account for the observed heterogeneity (OR, 2.282; 95% confidence interval [CI], 1.238–4.205) (Figure 2).

Additional analyses were run to exclude the role of a single large study effect, assess the consistency of cumulative outcomes over time, and evaluate potential publication bias (Figure 3). The one-study-removed analysis showed similar pooled outcomes without significant deviation from the overall result after removal of individual studies, suggesting no significant effect from a single large study (Figure 3A). Cumulative analysis showed temporal progression, demonstrating a statistically significant risk of SIBO as studies were added over time (Figure 3B). Potential publication bias was evaluated with a funnel plot, which showed 4 outlying studies with high standard error at the right margin of the plot (Figure 3C). The Duval and Tweedie trim-and-fill method was applied, resulting in 4 studies being imputed with an adjusted OR of 1.440 (95% CI, 0.778–2.667), showing no relationship between risk of SIBO and PPI use after accounting for publication bias.

A subgroup analysis was performed to examine the effects of SIBO testing modality, one of the presumed sources of heterogeneity, on the pooled results (Figure 4A). In the subgroup including only studies using duodenal/jejunal aspirate for SIBO diagnosis, an increase in risk for SIBO among PPI users was noted (OR, 7.587; 95% CI, 1.805–31.894). Among studies using GHBT for SIBO diagnosis, no relationship between PPI use and SIBO was seen (OR, 1.93; 95% CI, 0.69–5.42). Further division of the breath-test subgroup was not possible given the presence of only one study using the lactulose hydrogen breath test, and one study using the 14C-glycocholate breath test. Finally, when the Duval and Tweedie trim-and-fill method was applied to the subgroup of studies using duodenal/jejunal aspirate for SIBO diagnosis to account for potential publication bias, 2 studies were imputed with a resultant adjusted OR of 3.776 (95% CI, 1.144–12.464).

A second subgroup analysis was performed to assess the effects of study and control group design (Figure 4B). Studies using a self-control or pretest-posttest design, in which subjects were tested for SIBO before and after PPI exposure, showed an association between SIBO and PPI use (OR, 9.607; 95% CI, 1.646–56.089). On the other hand, studies using a separate group of PPI nonusers as controls showed no statistically significant risk for SIBO among PPI users (OR, 1.658; 95% CI, 0.869–3.163). Further analysis of the subgroups by stratification showed no change in the effect of SIBO testing modality on outcome when controlling for study design. However, when controlling for SIBO testing modality, no difference in SIBO risk among PPI users was seen by study design. These analyses suggest that the effect of test modality on outcome was independent of study design, but not vice versa.

Additional investigation of the effects of dose, duration, and type of PPI exposure on SIBO diagnosis could not be assessed as a result of insufficient data. Clinical indications for SIBO testing, such as irritable bowel syndrome, were not specified in every study, and therefore could not be included in further analysis.

Discussion

For the primary metameter of interest, risk of SIBO with PPI use, 11 studies were examined and evidence of increased risk was detected using random-effects pooled meta-analysis. Although there was significant heterogeneity in results among studies, 6 of 11 included studies reported a statistically significant increase in SIBO risk with PPI use. When potential publication bias was accounted for using the Duval and Tweedie trim-and-fill method, the adjusted OR was no longer statistically significant. Subgroup analysis showed an association between PPI use and SIBO when the diagnosis was made with duodenal/jejunal aspirate culture, the current gold standard. This association persisted even after adjusting for potential publication bias. In contrast, there was no relationship with PPI use when SIBO was diagnosed by GHBT. Although subgroup analysis by study type showed a limited association with SIBO and PPI use for both self- and separately controlled studies, there appeared to be some interaction between study type and testing modality. A review of the studies (Table 1) also showed that of the 5 studies using aspirate for SIBO diagnosis, 3 featured separate controls, whereas of the 7 studies with separate controls, only 3 were aspirate studies. In this case, association as a result of study type may account for part of the relationship between SIBO and PPI use seen with studies using aspirate culture as the testing modality.
This meta-analysis explored the relationship between PPI use and the risk of SIBO, an area that continues to remain controversial. Given the large patient population analyzed in this study, we believe that it contributes significantly to scientific knowledge and clinical care of patients taking PPIs. When a highly accurate diagnostic test (duodenal/jejunal aspirate culture) was used, PPI use appeared to increase the risk of SIBO. Along with studies linking other adverse events to chronic acid suppression, such as pneumonia, bone loss, and enteric infections, these results highlight the potential toxicity of PPIs, the need for judicious application, and the importance of medication reduction when reaching treatment goals.

The disparate findings between duodenal/jejunal aspirate culture and GHBT studies underscore the need for a clinically relevant and accurate outcome measure for the diagnosis of SIBO. The current gold standard is duodenal/jejunal aspirate culture.
which involves an invasive procedure such as endoscopy or fluoroscopy to intubate the small bowel to obtain duodenal/jejunal fluid. SIBO is diagnosed when bacterial counts exceed $10^5$ colony forming units per milliliter of fluid aspirate. Although the sensitivity and specificity of the test approach 100%,31 practical considerations such as patient discomfort and expense resulted in the introduction of breath testing for SIBO diagnosis. Breath testing relies on the ingestion of a test dose of carbohydrate, which can then lead to the release of a radioactive isotope (14C-D-xylose or 14C-glycocholate breath tests) or hydrogen species (glucose or lactulose hydrogen breath tests) when digested by a higher load of bacterial flora in SIBO patients. Although diagnostic breath tests for SIBO are simpler to administer, less invasive, and less costly than duodenal/jejunal aspirate culture, they are comparatively less sensitive and specific31,32 (Table 2). Potential contributors to the low sensitivities of breath testing include increased conversion of hydrogen to methane by certain gut microbes,33 recent ingestion of high-carbohydrate foods that can prolong hydrogen secretion, hyperventilation from recent exercise or pulmonary disease, increased loads of oral bacteria, short-gut syndrome, and low anaerobic bacterial load in the colon.34 Compounding the situation are the multiple thresholds of isotope, hydrogen, and methane detection that have been used to diagnose SIBO by breath testing. A systematic review of diagnostic testing options for SIBO published in 2008 showed no clear standard or validity of any of the testing modalities across 71 peer-reviewed publications.35 The relatively poor performance and lack of standardization of breath tests therefore have led many to question their validity and utility in the clinical diagnosis of SIBO.

In this meta-analysis, the absence of a clear association between PPI use and SIBO in the pooled analysis of studies using GHBT as a diagnostic modality may have resulted from misclassification of SIBO diagnosis owing to its low sensitivity and variable specificity, leading to inaccurate and inconsistent outcomes. One possible contributor to the observed inconsistency was the lack of a universally accepted cut-off threshold for many of the breath tests used to diagnose SIBO, even within a single testing modality. Such inconsistency likely detracts from the usability of GHBT and other diagnostic breath tests for SIBO diagnosis. Our data also highlighted the possibility that GHBT may be inadequate to accurately diagnose SIBO, especially as an outcome measure in research studies. Although duodenal/jejunal aspirate culture remains the gold standard for diagnosis of SIBO, its cost and invasiveness would likely prevent its widespread use in the clinical setting and for epidemiologic study. Therefore, further refinement of current breath testing modalities or development of a new, accurate, low-cost, low-invasive, rapid, and cost-effective method for SIBO diagnosis would be highly desirable.

**Table 2. Sensitivity and Specificity of SIBO Testing Modalities**

<table>
<thead>
<tr>
<th>Testing modality</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirate culture</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>14C-D-xylose breath test</td>
<td>14%–95%</td>
<td>100%</td>
</tr>
<tr>
<td>14C-glycocholate breath test</td>
<td>33%</td>
<td>76%</td>
</tr>
<tr>
<td>GHBT</td>
<td>6%–93%</td>
<td>78%–100%</td>
</tr>
<tr>
<td>Lactulose hydrogen breath test</td>
<td>6%–68%</td>
<td>44%–70%</td>
</tr>
</tbody>
</table>

from recent exercise or pulmonary disease, increased loads of oral bacteria, short-gut syndrome, and low anaerobic bacterial load in the colon.34 Compounding the situation are the multiple thresholds of isotope, hydrogen, and methane detection that have been used to diagnose SIBO by breath testing. A systematic review of diagnostic testing options for SIBO published in 2008 showed no clear standard or validity of any of the testing modalities across 71 peer-reviewed publications.35 The relatively poor performance and lack of standardization of breath tests therefore have led many to question their validity and utility in the clinical diagnosis of SIBO.

In this meta-analysis, the absence of a clear association between PPI use and SIBO in the pooled analysis of studies using GHBT as a diagnostic modality may have resulted from misclassification of SIBO diagnosis owing to its low sensitivity and variable specificity, leading to inaccurate and inconsistent outcomes. One possible contributor to the observed inconsistency was the lack of a universally accepted cut-off threshold for many of the breath tests used to diagnose SIBO, even within a single testing modality. Such inconsistency likely detracts from the usability of GHBT and other diagnostic breath tests for SIBO diagnosis. Our data also highlighted the possibility that GHBT may be inadequate to accurately diagnose SIBO, especially as an outcome measure in research studies. Although duodenal/jejunal aspirate culture remains the gold standard for diagnosis of SIBO, its cost and invasiveness would likely prevent its widespread use in the clinical setting and for epidemiologic study. Therefore, further refinement of current breath testing modalities or development of a new, accurate, low-cost, low-invasive, rapid, and cost-effective method for SIBO diagnosis would be highly desirable.

**Table 2. Sensitivity and Specificity of SIBO Testing Modalities**

<table>
<thead>
<tr>
<th>Testing modality</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirate culture</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>14C-D-xylose breath test</td>
<td>14%–95%</td>
<td>100%</td>
</tr>
<tr>
<td>14C-glycocholate breath test</td>
<td>33%</td>
<td>76%</td>
</tr>
<tr>
<td>GHBT</td>
<td>6%–93%</td>
<td>78%–100%</td>
</tr>
<tr>
<td>Lactulose hydrogen breath test</td>
<td>6%–68%</td>
<td>44%–70%</td>
</tr>
</tbody>
</table>
and noninvasive diagnostic test for SIBO is urgently needed for both clinical and investigative use.

The second subgroup analysis, which examined the possible role of study design, showed a statistically significant association between PPI use and SIBO in studies comparing the same cohort of patients before and after PPI exposure. On the other hand, studies comparing SIBO diagnosis in separate cohorts of patients with and without PPI exposure showed no association. These results may reflect the underlying pathophysiology and patient-specific nature of SIBO. Prior studies of duodenal/jejunal aspirate cultures showed that SIBO may be caused by increased levels of regular flora or bacterial species that have not yet been identified.36,37 Indeed, the present findings support the belief that the pathophysiology and exact bacterial composition of SIBO may be unique for each individual, thus leading to potential inconsistent outcomes when different groups of patients are compared against each other. In addition, although previous studies have identified several characteristics such as age, anatomy, and motility disorders that may predispose to SIBO,38 there are likely many other currently unknown factors that contribute to the alteration in intraluminal bacterial environment associated with SIBO. These undiscovered factors may introduce confounding to studies featuring 2 cohorts of subjects. Even in the case-control studies included, subjects could be matched only by demographics or known factors associated with SIBO, which may not be sufficient owing to the complexities of SIBO pathophysiology. Therefore, subgroup analysis of studies featuring subjects who served as their own controls, in which the intestinal flora was most comparable and unknown factors were least likely to contribute to the results, showed a more consistent and significant association between PPI use and the development of SIBO.

One limitation of this study was the detection of possible publication bias. The funnel plot (Figure 4) suggests the presence of 4 positive, outlying studies that were not balanced by negative studies. Additional investigations including reference review did not reveal any further peer-reviewed studies for inclusion. Although this may represent publication bias, it may also reflect a truly significant positive relationship between PPI and SIBO. Given the controversy in the field, the clinical utility and impact of even a negative study makes publication bias less likely. However, as stated earlier, the subgroup analysis of studies using duodenal/jejunal aspirate studies to detect SIBO revealed an association between PPI use and SIBO that remained significant even after adjusting for potential publication bias. Other expected limitations of meta-analyses, including lack of access to primary data, and covariates of interest that could not be accounted for individually, such as age of subjects and specific medical diagnoses, should be considered in the assessment of these findings. In addition, although they were strongly positive, the wide CIs detected in the subgroup analyses require care in further interpretation of the results.

Finally, caution should be taken in the translation of these results to clinical practice. Prior studies have shown significant overlap between symptoms associated with SIBO and many other functional disorders of the gastrointestinal tract, such as irritable bowel syndrome.39-41 However, results from SIBO testing have been inconsistent among patients with irritable bowel syndrome or functional gastrointestinal symptoms.42-44 In addition, variable response to antibiotic treatment for SIBO both in terms of symptoms and follow-up testing further highlights the complexity in the pathophysiology and epidemiology of gastrointestinal symptoms, functional and motility disorders, and SIBO. Therefore, a clear causal relationship between positive SIBO testing (including both duodenal/jejunal aspirate culture and breath testing) and gastrointestinal symptoms has not been firmly established. Because SIBO testing results, rather than clinical symptoms, have been the primary measured outcomes of this meta-analysis and most studies to date on the relationship between PPI and SIBO, it remains to be determined whether the observed increase in SIBO among PPI users actually correlates with significant clinical outcomes such as symptoms, malabsorption, or mortality.

In conclusion, this meta-analysis showed a statistically significant association between PPI use and SIBO, only when the diagnosis was made by a highly accurate testing modality (duodenal/jejunal aspirate culture). Studies using GHBT to diagnose SIBO did not result in a significant outcome. Prior conflicting outcomes in the published literature may have resulted from inconsistencies in the diagnostic methods and cut-off values used in those studies. Both clinicians and patients should be judicious in the use of PPI and consider dose-tapering whenever possible. In addition, a high level of suspicion should be raised when evaluating PPI users presenting with symptoms or signs suggestive of SIBO. Further studies are needed to refine the diagnostic modalities for SIBO, characterize the underlying pathophysiology of SIBO associated with acid suppression, and assess the impact of this relationship on clinical outcomes.

References
10. Hoffmann JC, Zeitz M. Small bowel disease in the elderly: diarr-

Reprint requests
Address requests for reprints to: Walter W. Chan, MD, MPH, Division of Gastroenterology, Brigham and Women’s Hospital, 75 Francis Street, Boston, Massachusetts 02115. e-mail: wwichan@partners.org; fax: (617) 525-0338.

Conflicts of interest
The authors disclose no conflicts.