LIMITATIONS OF ^{13}C-UREA BREATH TEST (UBT) AND STOOL ANTIGEN TEST (SAT) IN DIAGNOSIS OF *HELICOBACTER PYLORI* (HP) INFECTIONS

Synopsis

Helicobacter pylori (HP)(1) management (including diagnosis and therapy) has been exhaustively reviewed in several reports. These reports are unanimous in that several clinical conditions seriously hamper the diagnostic value of two HP tests: **^{13}C-Urea Breath Test (UBT) and Stool Antigen test (SAT),** because of the **false-negative** results frequently (up to 40%) obtained in these conditions. Basically, these false-negative results are due to decreased bacterial loads in the stomach mucosa, and include the following clinical conditions: 1) use of PPI medication; 2) use of antibiotics; 3) bleeding peptic ulcer; 4) atrophic gastritis (with or without intestinal metaplasia); 5) gastric cancer; 6) MALT lymphoma, and 7) partial gastrectomy. Since the late 1990's, it has been well established that UBT also gives **false-positive** results in cases where urease-producing bacterial species are colonizing an achlorhydric stomach due to atrophic gastritis or a long term use of proton pump inhibitors (PPIs).

It is to be emphasized that **neither UBT nor SAT** (or **HP serology**) is capable of diagnosing **atrophic gastritis** (AG), caused by HP infection or autoimmune disease, thus missing the patients at high risk for important clinical sequels of AG: i) gastric cancer, ii) esophageal cancer, iii) vitamin-B12 deficiency (due to malabsorption), and iv) malabsorption of calcium, iron, magnesium and certain medicines.

Conclusion: Because firmly documented and strongly emphasized, it is mandatory that these **serious limitations** (i.e., **false-negative** results in true disease, **false-positives** with no HP infection, and failure to diagnose AG) in use of UBT and SAT are properly acknowledged by all laboratories offering these two tests for diagnosis of HP infections also in Finland. Importantly, neither UBT nor SAT (or HP serology) is capable of diagnosing AG.

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^{13}C-UREA BREATH TEST (^{13}C-UBT)

Limitations

The UBT test has **serious limitations** and where the test results should be **interpreted with caution** (2-9). There is firm evidence to implicate that **recent use of proton pump inhibitors (PPI)** (within 2 weeks) or **antimicrobials** (within 4 weeks) may lead to a decrease in the gastric bacterial load causing **false-negative** results (10,11,12). **Bleeding** can also reduce the sensitivity of both UBT and SAT (10,11). Data from a systematic review suggests repeating diagnostic tests in patients with **bleeding ulcer** after at least 4 weeks in case of a negative result (13). In patients with **precancerous** conditions (e.g**. atrophic gastritis, intestinal metaplasia**) or gastric cancer, as well as in patients with **partial gastrectomy**, diagnostic tests may have lower accuracy (13).

The same limitations as acknowledged in the Italian Consensus Report (5) are also emphasized as limitations of the UBT and SAT tests in the latest Maastricht Consensus Report (3). It is clearly stated that significant decrease of the gastric HP load arises from the following conditions: **i)** use of **antimicrobial agents, ii)** use of **anti-secretory drugs (PPI), and iii) in bleeding ulcers**. Importantly, bacterial load may be **permanently low** in premalignant and malignant lesions, including i) **atrophic gastritis, ii) intestinal metaplasia (IM)** or **iii) MALT** (mucosal associated lymphatic tissue) **lymphoma** (14,15).

<u>Use of PPI medication</u>

In brief, several studies have shown that by increasing the gastric pH, **PPI use** leads to local changes in the stomach (10). Because PPI drugs have anti-microbial properties, the bacterial load decreases, especially in the antrum, causing **false-negative** results of the UBT tests, in contrast to serology that remains unaffected. Most of these studies have been carried out with UBT and showed a **10-40% rate of false-negative results** (16,17). In addition to PPI treatment (10,11,12), also **H2-blockers** may lead to some **false-negative** results of UBT, but to lesser extent than PPI medication (19,20).

<u>Antimicrobials</u>

As to the use of **antimicrobials**, the evidence is notwithstanding that the local bacterial load of HP in the stomach will be reduced, leading to potentially false negative UBT results, as stated in the Consensus reports, based on several well documented studies (3,5,10,11,12,21,22). Thus, Perri et al. (21) performed UBT in 41 HP-infected subjects before and after 1 day of therapy with (among other drugs) amoxycillin (2.5 g). They showed that even a short course of drugs specific for HP may result in a false-negative UBT (21). The authors concluded that false-negative results are likely even after 1 day of therapy with bactericidal or anti-secretory (PPI) drugs. In another recent study, Leung et al. examined the serial changes of UBT results in 35 hospitalized patients who were given antibacterial therapy for predominantly chest and urinary infections (22), most (91 %) receiving a single antibiotic of either a penicillin or cephalosporin group. Serial UBTs were performed within

24 hours of initiation of antibiotics, at one-week and at six-week post-therapy. The results showed that one-third of HP-infected individuals had transient **false-negative** UBT results during treatment with antibacterial agent, albeit a full clearance of HP infection by regular antibiotic consumption was very rare (22).

Bleeding ulcer

As to the accuracy of UBT in cases of **bleeding ulcers**, this topic was subjected to a comprehensive meta-analysis recently (13). It has been suggested that prevalence of HP in **peptic ulcer bleeding** (PUB) is lower than that in non-complicated ulcers. These authors performed a systematic review of studies assessing the prevalence of HP infection in patients with PUB, including 71 articles, with 8,496 tested PUB patients. In meta-regression analysis, the **UBT** was **reliable only** when **delayed until at least 4 weeks after the PUB episode** (13,24,25).

In areas of low HP infection prevalence, a test-and-treat strategy should be considered (3,5). In the setting of **peptic ulcer bleeding**, histology and rapid urease test maintain a high specificity, but are affected by **a low sensitivity**, possibly leading to under-treatment (13,23). Importantly, serology seems not to be influenced by upper gastrointestinal (GI) bleeding (2,6,7).

Cancer precursors: atrophic gastritis (AG) and intestinal metaplasia (IM)

Atrophic gastritis (AG) is another clinical condition to be associated with substantial proportion of **false-negative** UBT results. This subject has been studied in detail by Kokkola et al. (15,26,27). In their first study, patients with atrophic corpus gastritis (AGC) and elevated HP antibody titers but HP-negative UBT and histology, were randomized into eradication therapy or follow-up only (15). HP antibody levels decreased significantly in six out of seven patients in the eradication group, while in the follow-up group, the titers declined in only one out of eight patients. In patients with AGC, positive HP serology results may indicate an ongoing infection in spite of **negative UBT** and histology results.

In another study, these authors made a direct comparison of UBT, HP serology and histology in 50 male patients with **AGC** (26). The results are revealing: HP was detected in 15 (30%) patients by histology and in 14 (28%) by UBT, whereas increased serum HP-antibody levels were found in 41 (82%) patients (p<0.0001). HP infection was associated with AGC in 84% of the present patients. The authors concluded that in patients with **AG and IM**, prevalence of HP infection will be **underestimated** if only **UBT** (and biopsy-based diagnostic methods) are used (26).

Similar conclusions were drawn in another recent study by Lahner et al. (28), who examined 27 AGC patients using UBT and SAT, to assess whether the diagnostic yield of HP in AGC could be increased by these two tests, as related to histology alone. Without going into the details, the results implicated that **in AGC patients, neither the UBT nor SAT added any useful information** regarding HP infection, but a combination of histology and serology are needed to define the HP status among AGC patients (28). Indeed, when followed-up for long enough, even HP serum

antibodies disappear spontaneously within 10 years in almost one fourth of the patients with advanced AGC. This disappearance of HP antibodies is accompanied by no or more than a mild improvement of the gastric mucosa (27).

MALT lymphoma

MALT (mucosal-associated lymphatic tissue) **lymphoma** is another specific condition, ascribed to HP infection (3,5), but known to be associated with reduced bacterial load and thus susceptible to **false-negative UBT** results (2,3,5,6,7). Because gastric MALT lymphoma is a rare disease, few studies comparing the accuracy of diagnostic tests in this group of patients have been carried out, and only a limited number of tests (essentially histological) were performed (14). In one of those few studies, a total of 90 patients with low-grade gastric MALT lymphoma were enrolled, comparing histology, serology PCR and culture. Histology (97.5%) and serology (95.0%) were the two most sensitive tests, far superior to the other HP tests (14).

Importantly, **HP-negative MALT lymphomas** exist, testing repeatedly HP-negative   (29). In the search for HP based on histology and **the UBT**, there are cases with a series of **false-negative results**, thus confirming the possibility of a lower detectability of HP in patients with MALT gastric lymphoma and supporting the use of additional tests in diagnosis. Although patients with gastric MALT lymphoma with no HP are less responsive to HP eradication, a portion of the HP-negative cases are potentially curable by HP eradication therapy alone. Although the rationale for this finding is not fully elucidated, it is suspected that **some HP-negative cases** of MALT lymphoma are **false-negatives** due to patchy distribution of the microorganism in the gastric mucosa and limited tissue sampling during biopsy. In addition, PPI therapy before biopsy reduces the sensitivity of HP detection, and PPI should be discontinued at least 2 weeks before HP testing. The patient's symptoms may disappear and HP become undetectable with **a negative UBT**, if the therapy is effective, but, importantly, histological response usually lags behind the HP eradication, and a lymphoma infiltrate may persist up to 12 months or even longer. In contrast to serology, the UBT may produce **false-negative** results if performed after the use of HP- and urease-suppressive therapies, such as PPIs and antibiotics (30).

False-positive results with UBT

Apart from false-negative results listed above, UBT also gives **false-positive** results, which have received more attention during the past 10 years (31,32,33,34). These false-positive results are typical to patients with achlorhydric stomach (due to AG or a long term use of PPIs), where **urease-positive** bacterial species (33,34) or yeast-like organisms (31) colonize.

In fact, however, this possibility of **false-positive UBT results** was well **known already in the late 1990's**, when e.g. the future Nobel Laureate Barry Marshal describes (in his Chapter to the Textbook of Lee & Megrau, **1996**), that **false-positive** breath test results have been reported in gastrectomy, generally related to the presence of **urease-positive bacteria other than H. pylori** (35). The 13C-UBT was positive and urease-positive bacteria other than H. pylori were recovered in

gastric juice in hypochlorhydric children due to PPI use, reported by Michaud et al. in **1998** (36). We know now, that gastric bacterial overgrowth is a constant phenomenon in hypochlorhydria (31). Already in the late 1990's, it was shown that the number of bacteria on stomach mucosa is comparable to that of gastric juice (37), and these **non-H. pylori bacteria** are also found embedded in the mucus and even in close contact with gastric microvilli, similar as H.pylori per se (38).

In their study, Gurbuz et al. (32) compared UBT in the detection of HP infection with histology and the rapid urease test (RUT). Histology revealed dense yeast-like micro-organisms in the biopsy specimens in **all patients with false-positive results** by UBT, making the authors to conclude gastric mucosal colonization by **yeast-like micro-organisms** with urease activity can account for the high frequency of false-positive results for UBT (32).

Brandi et al (33) evaluated the presence of urease-positive bacteria other than HP in gastric juice and mucosa in 25 hypochlorhydric and 10 control subjects. Altogether, 6 hypochlorhydric patients had 10 strains of urease-positive non-HP bacteria, among which *Staphylococcus capitis urealiticum* showed the strongest urease activity. The authors concluded that hypochlorhydric patients present many urease-positive bacteria other than H. pylori. The strong urease activity may be responsible for false-positive results at UBT test in patients with suspected HP-infection (33).

In another study, the UBT gave false-positive results in 4/102 subjects, shown to be caused by the presence of **urease-positive bacteria** in the oral cavity and stomach. Altogether, 5 bacterial species with urease activity (*Proteus mirabilis, Citrobacter freundii, Klebsiella pneumoniae, Enterobacter cloacae and Staphylococcus aureus*) were isolated from the oral cavity and/or stomach. Because all of the patients with a false-positive UBT result were suffering from AG, it is obvious that the false-positive results in the UBT are a result from colonization of urease-positive bacteria in hypochlorhydric/achlorhydric stomach (34).

STOOL ANTIGEN TEST (SAT)
In most occasions listed above for the UBT, the same limitations and potential sources of error apply to the SAT as well.

Limitations
Due to the same inherent reason, i.e. low bacterial load in the stomach, a **recent use of PPIs** (within 2 weeks) or **antimicrobials** (within 4 weeks) also contributes to **false-negative** results in the **SAT** (10,11,12). Similarly, bleeding can also reduce the sensitivity of the SAT (10,11). Data from a systematic review suggests repeating the SAT in patients with **bleeding ulcer** after at least 4 weeks in case of a negative result (13). In patients with **precancerous** conditions (e.g**. atrophic gastritis**) or gastric cancer, as well as in patients with **partial gastrectomy**, diagnostic tests may have lower accuracy (13).

As discussed for the UBT, several studies have shown that by increasing the gastric pH, PPI use leads to reduced bacterial load in the stomach, especially in the antrum, causing **false-negative** results of the diagnostic tests, **with the exception of HP serology** (3,5). Albeit most of these studies have been carried out with UBT, showing 10-40% rate of false-negative results (16,17), similar results have been obtained with the SAT as well (41,42). Interestingly, the same bias seems to affect also the biopsy-based tests (culture, rapid urease test and histology)(43).

CAVEATS OF RELYING ON UBT AND SAT IN DIAGNOSIS OF HP INFECTION
The impact of the consequences that potentially can result from a false-negative UBT or SAT in diagnosis of HP infection depends on the clinical condition where the false-negative test results have been relied on, and whether corrective measures have been taken or not. Similarly, the development of these sequels can be **more or less protracted** depending on whether i) HP infection alone (with no other gastric pathology) has been missed by the false-negative test, or ii) whether HP infection has already progressed to the stage of clinical sequels (e.g. AG, IM or early cancer) at the time of the false-negative UBT or SAT result. Obviously, the error is less critical in cases of HP-associated superficial gastritis where the diagnosis is missed due to the use of antibiotic or PPI medication. In cases with bleeding ulcer, the diagnosis is usually reached by clinical means and not primarily by HP testing. However, missing **any of the premalignant gastric lesions** (AG, IM, dysplasia) or gastric malignancy (GC or MALT lymphoma) because of the wrong diagnostic test (UBT, SAT) was used without confirmatory methods (HP-serology, histology) must be considered as a serious unprofessional conduct (malpractice). This is best avoided by recognizing the caveats and potential sources of errors in using UBT and SAT in diagnosis of HP infection under these special circumstances. The false-positive UBT results lead to over-treatment and unnecessary control testing or even gastroscopies among subjects with no true HP infection.

Finally, it should be emphasized that **none of the three tests**: UBT, SAT or HP serology, is capable of diagnosing **atrophic gastritis** (AG), caused by HP infection or autoimmune disease (44,45,46). Thus, all these tests will fail to find **the patients at high risk** for important clinical sequels of AG: i) gastric cancer, ii) esophageal cancer, iii) vitamin-B12 deficiency (due to malabsorption), and iv) malabsorption of calcium, iron, magnesium and certain medicines. Apart from the gastric and esophageal cancer, of particular concern are the patients with AG who develop malabsorption of vitamin-B12 and calcium, with potential to serious clinical complications, including pernicious anemia, dementia, Alzheimer's disease, peripheral neuropathy as well as osteoporosis.

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