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Lewis and Secretor status and *Helicobacter pylori* eradication

To the Editor:

In the paper by Matsuo et al. [1] recently published in *Epidemiology and Infection*, the authors stated that *Le* gene polymorphisms might affect the success of *H. pylori* eradication. We obtained different results when we evaluated *H. pylori* infection, host Lewis phenotype/genotype, FUT2 gene polymorphisms and secretor status in a sample of adults from the north of Portugal [2].

A total of 174 individuals with biopsy-documented *H. pylori* infection were randomly assigned to one of two eradication schemes (treatment A: omeprazole, tinidazole and clarithromycin; treatment B: omeprazole, amoxicillin and clarithromycin). *H. pylori* eradication was verified by urea breath test. In our study, the overall rate of eradication was higher (86·2%) than reported by Matsuo et al. (61·3%) [1]. Eradication was more frequent in participants above 45 years of age (94·6% *vs.* 80·0%, $P<0·05$) but no significant differences were observed according to treatment regimen (86·7% *vs.* 84·9%) or Lewis blood group phenotype (86·3% *vs.* 86·8%).

We evaluated three Le inactivation polymorphisms (T202C, G508A and T1067A) by direct sequencing [2] in 17 participants with a blood phenotype Le^{a-b-}. Infection remained in three cases: one was homozygous for G508A and two were homozygous for T1067A. In the remaining 14 participants in whom eradication was successful, only three were homozygous for inactivating polymorphisms (G508A). In contrast to Matsuo et al. [1], our results do not suggest an association between Le gene polymorphisms and eradication efficiency.

We previously reported that 80% of individuals homozygous for Le-inactivating polymorphisms express Lewis b in the gastric epithelium [2], probably due to the presence of another α1,4-fucosyltransferase (FucT V or a still unidentified enzyme), showing that the Le genotype may not provide the right information about the Lewis phenotype in the gastric mucosa.

A FUT2 gene polymorphism (G428A) was evaluated by *Ava*II restriction and the secretor status was studied in gastric tissue sections using the lectin UEA1. From 80 cases evaluated for FUT2 gene polymorphism, eradication was observed in 84·6% of those homozygous for G428A and in 85·4% of those that had a normal FUT2 gene.

Secretor status and *H. pylori* eradication were determined in 169 individuals and eradication was observed in 81·0% of non-secretors, 86·7% of those UEA1 weakly positive, and 87·5% of those UEA1 strongly positive. No significant association was observed between the secretor status or G428A FUT2 polymorphism and *H. pylori* eradication. We further observed that 53·8% of the homozygous individuals for the inactivating FUT2 polymorphism (G428A) were secretors as evaluated by UEA1. This observation can be partly explained by the expression of Lewis y in the superficial gastric epithelium, which can be recognized by UEA1.

The same Japanese group showed in a previous work [3] that individuals carrying a normal Le gene were less susceptible to infection by *H. pylori* than individuals with an inactive Le gene. In our study 82 individuals were not infected by *H. pylori*, and none was Le^{b-} and non-secretor [2].

Conflicting results between our survey and the studies by Matsuo et al. [1], and Ikehara et al. [3], show that the *in vivo* role played by recognition of host Lewis b and/or H type-1 antigens by BabA adhesin from *H. pylori* is far from clarified. Extended studies accounting for the Lewis phenotype/genotype, secretor status/FUT2 polymorphisms, and BabA adhesion status from *H. pylori* are needed to understand the whole scenario played by glycosylated structures

on *H. pylori* infection *in vivo*, and to depict all of the complexity of the host–agent interaction in human *H. pylori* infection.

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The authors reply:

Serpa et al. evaluated the association between the *Helicobacter pylori* (*Hp*) eradication rate and the Lewis/Secretor phenotype and found 17 participants within their study cohort to be Lewis^{a-b-}, but only six of them were homozygous for *le*/*le* (*le1*/*le1 and le2*/*le2*) regarding *FUT3*/*FucT-III* (*Lewis; Le*) gene polymorphisms. We conclude: (i) they failed to find an association between the inactive *FUT3* genotype and the eradication rate; (ii) as a possible reason why *Le* gene polymorphisms might not be reflected in efficient eradication therapy, they pointed to Le^b antigen expression detectable on surface gastric mucosa from Lewis^{a-b-} blood phenotype patients; (iii) moreover, regarding the frequency of non-secretor phenotype evaluated by lectin-histochemstry using *Ulex europaeus* agglutinin 1 (UEA1) on biopsy samples from gastric mucosa, they found an association neither between the UEA1 result and the eradication rate nor between UEA1 and the efficiency of *Hp* eradication therapy. As UEA1 binds specifically to α1,2-linked fucose expressed on gastric mucosa, a lack of UEA1 binding suggests homozygotes for inactivated alleles of *FUT2*/*Secretor* (*Se*) *gene* by G428A nonsense mutation (*se1*). The inconsistency between our results and theirs warrants further studies.

Before drawing firm conclusions, several issues must be considered. First, Serpa et al. genotyped only subjects with Le^{a-b-} defined by blood phenotyping. As discussed in our and other previous reports, the blood phenotype test, a haemagglutination test, is vulnerable to mistyping [1]. Lewis blood antigens on erythrocytes are not synthesized by erythrocytes themselves but are acquired from plasma by adsorption [2], which is a subject influenced by certain biological conditions. Yazawa et al. [3] described subjects with salivary α1,4-fucosyltransferase activity exhibiting Le^{a-b-} blood phenotype. Serpa et al. also found individuals with Lewis negative blood but positive gastric mucosa [4]. Thus, the fact that Serpa et al. found only 6 out of 17 individuals homozygous for *le* suggests that the other 11 individuals might be Lewis positive with uncertain medical conditions influencing Lewis antigen absorption steps. It might be advisable to reanalyse the eradication efficiency in their treatment cohort based on FUT3 genotyping of all the subjects.

Secondly, they suggested the possible existence of enzymes with α1,4-fucosyltransferase activity such as Fuc-T V based upon their finding that subjects with homozygous Lewis-inactivating polymorphisms express Lewis b antigen in the gastric epithelium [4]. Although false positive immunohistochemical findings need to be ruled out, we agree that this is a possibility. For confirmation, we recommend an evaluation of α1,4-fucosyltransferase activity itself in the gastric mucosa. Because Fuc-T V mRNA expression was not seen in gastric mucosa in our previous study [5], the presence of other fucosyltransferases should be investigated. Recently, *FUT10* and *FUT11* have been reported without information about their activities [6]. As the putative amino-acid sequences are similar to those of the α1,3-fucosyltransferase family, α1,4-fucose transfer activity to H type I to synthesize Le^b antigen might be exhibited, as with FUT3. It would provide an important finding if they succeeded in confirming Le^b carbohydrates in samples from Lewis negative gastric mucosa using MALDI–TOF MS or NMR.

Thirdly, they reported that the secretor status of gastric epithelium, defined by lectin-histochemistry using UEA1, did not correlate with *Hp* eradication

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rate and this is in agreement with our observations [7]. We also consider UEA1 lectin staining to be a valuable approach. Actually, homozygotes of the *FUT2* null allele (G428A nonsense mutation) in Caucasians, *se1*/*se1*, would not be expected for Japanese subjects, because the *sej* allele is common in east Asians, including Japanese, which still have 3% enzyme activity compared to wild-type alleles [8]. We often observed aberrant and partial Le^b expressions in surgically resected specimens from patients with *sej*/*sej* [A385T missensee mutation, the other name *sew* (*se* weak)]. Thus, we focused on *Se* allele polymorphisms for host factors against *Hp* infection in a previous study.

In addition, one further issue in the design of the two studies should be considered. We applied an *Hp*-IgG antibody as a marker of infection and eradication while colonization of *Hp* was used in their study. The large difference in *Hp* eradication rates observed might be explained to some extent by this difference.

Finally, we always encounter difficulty in obtaining consistent results across many studies using genetic polymorphisms [9]. In this sense, the proposal of Serpa et al. does make sense. A replication study with a larger, hopefully multi-ethnic population, is to be recommended.

We thank Serpa et al. for their interest in our study.

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A note on vaccination against meningococcal meningitis in infants

To the Editor:

Following the publication of our article [1] on the effects of various vaccination schedules on the time-course of outbreaks of meningococcal disease, it was drawn to our attention by a reader of this journal that a particular statement concerning vaccine efficacy may have not been entirely accurate. The statement in question was 'there exist capsular polysaccharide vaccines for the serogroups A, C, W-135 and Y, but these are not indicated for children below 2 years of age, because they are poorly effective in this age group'. According to the reader, 'Every year, tens of thousands of infants and young children in Africa and other countries contract meningococcal meningitis that is preventable with cheap, safe, available vaccines that are highly effective when administered as directed.'

Upon drawing the attention of these contradictory claims to the Editor of this journal, the matter was examined further by qualified referees. The disputed statement in ref. [1] was found to be valid for group C (which was the main focus of our paper), as the group C polysaccharide vaccine can induce tolerance in infants, resulting in a diminished response to a follow-up dose. However, our statement indeed suggested that the vaccine was inappropriate for groups A, W135 and Y as well. For group A, it seems that the statement may be incorrect [2], but this is not certain. For groups W135 and Y, a definite uncertainty exists. Clearly further studies are required to clarify this matter which has important consequences for the control of infant meningococcal meningitis.

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