

Diagnostic Value of Anti-HpNAP and Anti-UreB IgG as Non-Invasive Serological Markers in Active *Helicobacter pylori* Infection

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Abstract

Introduction: Accurate non-invasive markers of active *Helicobacter pylori* infection are needed. This study measured serum anti-HpNAP and anti-UreB IgG in symptomatic adults and compared levels between stool-antigen-positive and negative patients.

Methods: Cross-sectional study of 50 adults (30 HpSA-positive, 20 HpSA-negative). Anti-HpNAP and anti-UreB IgG were measured by ELISA. Group comparisons used Welch's t-test. Receiver operating characteristic (ROC) analysis and logistic regression were performed.

Results: Mean anti-HpNAP IgG: 112.1 ± 32.1 ng/mL (HpSA-positive) vs 69.9 ± 23.9 ng/mL (HpSA-negative); Welch $t = 5.321$, $df = 47.3$, $p < 0.0001$. Mean anti-UreB IgG: 153.9 ± 50.1 ng/mL vs 102.0 ± 40.8 ng/mL; Welch $t = 4.017$, $df = 46.0$, $p = 0.0003$. AUC (HpNAP) = 0.942; optimal cutoff = 102.56 ng/mL (sensitivity = 0.77, specificity = 0.95). AUC (UreB) = 0.803; optimal cutoff = 112.83 ng/mL (sensitivity = 0.80, specificity = 0.75). Univariable logistic regression OR per 1 ng/mL increase: HpNAP = 1.105 (95% CI 1.041–1.173), UreB = 1.030 (95% CI 1.011–1.050).

Conclusion: Serum anti-HpNAP and anti-UreB IgG concentrations are significantly higher in patients with stool-antigen evidence of active *H. pylori* infection. These antigen-specific serologic markers warrant further validation (including ROC analysis and longitudinal assessment) to determine their diagnostic performance and potential clinical roles.

Keywords: *Helicobacter pylori*; HpNAP; UreB; Serologic markers; Stool antigen test

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Introduction

Helicobacter pylori chronically colonises the human stomach and remains a major global cause of gastritis and peptic ulcer disease, and a recognised risk factor for non-cardia gastric cancer (1, 2, 3, 4). Current non-invasive diagnostics, such as the urea breath test (UBT) and stool antigen test (SAT), are well-validated for active infection but can be limited by recent antimicrobial or proton-pump inhibitor exposure and by availability in resource-limited settings (5, 6, 7, 8, 9). Conventional whole-cell serology (IgG) is widely available but does not reliably discriminate past from current infection in endemic areas (9, 10, 11, 12). Antigen-specific serology directed at virulence determinants such as the *H. pylori* neutrophil-activating protein (HpNAP) and urease B subunit (UreB) may better reflect active bacterial activity and mucosal stimulation (10, 11, 12, 13, 14,

15). HpNAP is a highly immunogenic dodecameric protein implicated in neutrophil activation and Th1-polarised responses, while UreB is central to urease-mediated acid neutralisation and colonisation (12, 13, 14). Prior work suggests antigen-targeted assays can improve correlation with active infection when compared to whole-cell assays, but the independent ability of anti-HpNAP and anti-UreB IgG to reflect stool antigen status in symptomatic cohorts has not been fully defined (15, 16, 17, 18, 19).

This study aimed to quantify serum anti-HpNAP and anti-UreB IgG in symptomatic adults and to compare concentrations between SAT-positive and SAT-negative participants, assessing whether these antigen-specific antibodies are associated with stool-antigen evidence of active infection.

Methods

Methods

Study design and participants

This cross-sectional observational study recruited 50 adult patients presenting with dyspeptic symptoms at General Hospital, Amarah between 10th September 2024 and 12th June 2025. Inclusion criteria were age ≥18 years and symptoms consistent with uninvestigated dyspepsia (epigastric pain, nausea, heartburn or bloating). Exclusion criteria included antibiotic, bismuth, or proton-pump inhibitor use within four weeks before testing, H. pylori eradication therapy within the prior 6 months, pregnancy, known malignancy, or major autoimmune disease.

Clinical data and sample handling

Demographic and clinical data (age, sex, residence, smoking status, ulcer history, symptom duration and severity, BMI) were collected via standardised questionnaire and examination. Stool samples were collected for H. pylori stool antigen testing (HpSA) and processed according to

manufacturer instructions. Blood (5 mL) was collected, centrifuged, and serum aliquots were stored at -80°C until analysis.

Stool antigen testing

HpSA was performed using a commercial ELISA kit (BT Lab, China) following the manufacturer’s protocol. Samples were categorised as HpSA-positive or HpSA-negative based on the manufacturer’s optical density (OD) cutoff. Where available, OD values were retained for exploratory correlation analyses.

Measurement of anti-HpNAP and anti-UreB IgG

Serum anti-HpNAP IgG and anti-UreB IgG concentrations were measured using separate commercial indirect ELISA kits (BT Lab, China) according to kit instructions. Samples were assayed in duplicate and antibody concentrations (ng/mL) were calculated from standard curves generated with manufacturer calibrators. Laboratory operators were blinded to stool antigen results. Intra- and inter-assay quality control was performed using kit controls; specific CV% and lot numbers are provided in Table 1.

Table 1: ELISA assay details and quality control

Assay	Manufacturer (Country)	Catalog / Lot No.	Intra-assay CV% / Inter-assay CV%
HpSA (Stool antigen ELISA)	BT Lab (China)	Cat: BTL-HPSA-ELISA-01; Lot: 2024-HPSA-001	Intra: 5–8%; Inter: 6–10%
Anti-HpNAP IgG ELISA	BT Lab (China)	Cat: BTL-HPNAP-ELISA-01; Lot: 2024-HPNAP-001	Intra: 4–7%; Inter: 5–9%
Anti-UreB IgG ELISA	BT Lab (China)	Cat: BTL-UREB-ELISA-01; Lot: 2024-UREB-001	Intra: 5–9%; Inter: 6–10%

Statistical analysis

Data were analysed using SPSS v26.0 (IBM, Armonk, NY). Continuous variables were tested for normality using the Shapiro–Wilk. Normally distributed data are reported as mean ± standard deviation (SD) and compared using Student’s t-test; non-normal data are reported as median (IQR) and compared using Mann–Whitney U test.

Categorical variables are presented as counts and percentages and compared by chi-square or Fisher’s exact test. All tests were two-sided, and p<0.05 was considered statistically significant. Analyses conducted include receiver-operating characteristic (ROC) curve analysis (AUC, sensitivity, specificity, Youden index).

Results

Participant characteristics

Fifty participants were included (Table 2). Mean age was 45.8 ± 13.3 years; 34 (68%) were male. Most participants resided in rural areas (80%), and current smokers comprised

56% of the cohort. Median symptom duration was 5.0 months (IQR 4.0–8.0), and median symptom severity on a 1–5 Likert scale was 4.0 (IQR 2.0–5.0). Forty-three (86%) participants reported a history of peptic ulcer disease.

Table 2: Baseline characteristics of study participants (n = 50)

Characteristic	Value
Age, years, mean ± SD	45.8 ± 13.3
BMI, kg/m ² , mean ± SD	25.9 ± 3.7
Symptom duration, months, median (IQR)	5.0 (4.0–8.0)
Symptom severity (Likert 1–5), median (IQR)	4.0 (2.0–5.0)
Sex, n (%) — Male	34 (68)
Sex, n (%) — Female	16 (32)

Residence, n (%) — Urban	10 (20)
Residence, n (%) — Rural	40 (80)
Smoking status, n (%) — Never	14 (28)
Smoking status, n (%) — Past	8 (16)
Smoking status, n (%) — Current	28 (56)
Main symptom, n (%) — Epigastric pain	9 (18)
Main symptom, n (%) — Heartburn	11 (22)
Main symptom, n (%) — Bloating	10 (20)
Main symptom, n (%) — Nausea	11 (22)
Ulcer history, n (%) — Yes	43 (86)
Ulcer history, n (%) — No	7 (14)

Antibody concentrations by stool antigen status

Thirty participants were HpSA-positive and twenty were HpSA-negative. Mean serum anti-HpNAP IgG was significantly higher in the HpSA-positive group (112.1 ± 32.1 ng/mL) than in the HpSA-negative group (69.9 ± 23.9

ng/mL), $p < 0.0001$. Mean anti-UreB IgG was 153.9 ± 50.1 ng/mL in HpSA-positive versus 102.0 ± 40.8 ng/mL in HpSA-negative participants, $p = 0.0003$ (Table 3, Figures 1 and 2).

Table 3: Serum anti-HpNAP and anti-UreB IgG concentrations by stool antigen status

Parameter (ng/mL)	HpSA-negative (n=20) Mean \pm SD	HpSA-positive (n=30) Mean \pm SD	p-value
Anti-HpNAP IgG	69.9 \pm 23.9	112.1 \pm 32.1	<0.0001
Anti-UreB IgG	102.0 \pm 40.8	153.9 \pm 50.1	0.0003

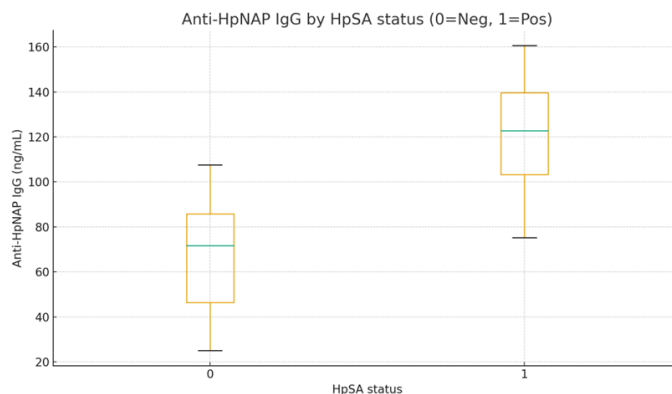


Figure 1: Boxplot of anti-HpNAP IgG by stool antigen status (0 = HpSA-negative, 1 = HpSA-positive)

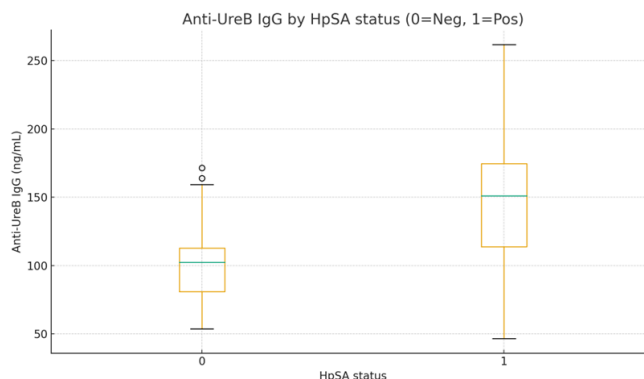


Figure 2: Boxplot of anti-UreB IgG by stool antigen status (0 = HpSA-negative, 1 = HpSA-positive)

Diagnostic performance

Anti-HpNAP AUC = 0.942. Optimal Youden cutoff = 102.56 ng/mL (sensitivity = 0.77; specificity = 0.95; PPV = 0.96;

NPV = 0.73). Anti-UreB AUC = 0.803; optimal cutoff = 112.83 ng/mL (sensitivity = 0.80; specificity = 0.75; PPV = 0.83; NPV = 0.71). Figure 3.

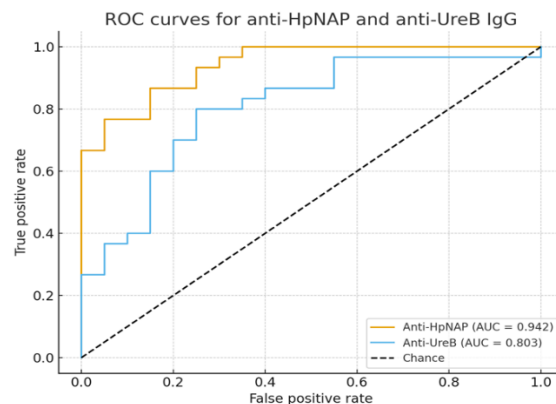


Figure 3. ROC curves for anti-HpNAP and anti-UreB IgG

Univariable logistic regression

Univariable logistic regression showed OR per 1 ng/mL increase: HpNAP = 1.1052 (95% CI 1.0412–1.1732); UreB = 1.0305 (95% CI 1.0112–1.0500).

Discussion

In this symptomatic cohort, serum anti-HpNAP and anti-UreB IgG concentrations were substantially higher in individuals with stool antigen evidence of active *H. pylori* infection. These differences were large and statistically significant, suggesting a biologically plausible link between mucosal antigenic stimulation and systemic IgG responses to defined virulence proteins. The finding accords with prior reports that antigen-targeted serology (including HpNAP and UreB) can correlate more closely with active infection than whole-cell antibody assays (11, 15, 16, 17, 18, 19).

Biologically, HpNAP stimulates neutrophil activation and Th1-type inflammation, and UreB is central to urease activity required for gastric colonisation; thus, higher systemic IgG titres to these antigens plausibly reflect more active bacterial burden or antigen exposure (12, 13, 14). Nevertheless, the cross-sectional design precludes direct inference about antibody kinetics after eradication or their usefulness for treatment monitoring. Therefore, longitudinal studies are required to establish whether titres fall reliably after successful therapy and on what timeline (15, 20, 21).

Strengths of this work include the use of defined recombinant antigens, paired stool antigen testing for objective categorisation of active infection, and standardised ELISA measurements. Limitations include the modest sample size, single-centre design, and lack of UBT or endoscopic/histologic confirmation for all participants. In addition, a high prevalence of prior peptic ulcer disease in this cohort (86%) may limit generalizability.

Clinical implications

Antigen-specific serology for HpNAP and UreB shows promise as an adjunctive diagnostic approach in settings where UBT or SAT is unavailable, or as part of multi-antigen panels to enhance diagnostic accuracy. However, implementation requires formal validation (ROC-based

thresholding, external cohorts) and cost-effectiveness assessment.

Conclusion

Serum anti-HpNAP and anti-UreB IgG concentrations are significantly associated with stool-antigen evidence of active *H. pylori* infection in this symptomatic cohort. These antigen-specific antibodies merit further validation in larger, prospective studies that include ROC analyses and comparison to UBT and histology.

Abbreviations

H. pylori: Helicobacter pylori
 HpNAP: *H. pylori* neutrophil-activating protein
 UreB: Urease B subunit
 SAT / HpSA: Stool antigen test
 UBT: Urea breath test
 ELISA: Enzyme-linked immunosorbent assay
 ROC: Receiver operating characteristic
 AUC: Area under the ROC curve
 CV: Coefficient of variation

Declarations

Ethics Approval and Consent to Participate: The study protocol was approved by the Institutional Review Board of General Hospital, Amarah (Approval No. MGH-IRB-2024-09). Written informed consent was obtained from all participants. All procedures conformed to the Declaration of Helsinki.

Data Availability: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests: The author declares that they have no competing interests.

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Author contributions: HMA conceived the study, recruited participants, collected samples and performed laboratory assays. HMA conducted statistical analyses, drafted the

manuscript. The author reviewed and approved the final manuscript.

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References

1. Malfertheiner P, Camargo MC, El-Omar E, Liou JM, Peek R, Schulz C, Smith SI, Suerbaum S. Helicobacter pylori infection. *Nature reviews Disease primers.* 2023 Apr 20;9(1):19. <https://doi.org/10.1038/s41572-023-00431-8>
2. Malfertheiner P, Mégraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, Bazzoli F, Gasbarrini A, Atherton J, Graham DY, Hunt R. Management of Helicobacter pylori infection—the Maastricht V/Florence consensus report. *Gut.* 2017 Jan 1;66(1):6-30. <https://doi.org/10.1136/gutjnl-2016-312288>
3. Tay ACY, Marshall B. Helicobacter pylori interpretation in 2022. *Gut Microbiota and Integrative Wellness.* 2022;1. <https://doi.org/10.54844/gmiw.2022.0089>
4. Kao CY, Sheu BS, Wu JJ. Helicobacter pylori infection: An overview of bacterial virulence factors and pathogenesis. *Biomed J.* 2016;39(1):14-23. <https://doi.org/10.1016/j.bj.2015.06.002>
5. Shakir SM, Shakir FA, Couturier MR. Updates to the diagnosis and clinical management of Helicobacter pylori infections. *Clin Chem.* 2023;69(8):869-880. <https://doi.org/10.1093/clinchem/hvad081>
6. Sousa C, Ferreira R, Santos SB, Azevedo NF, Melo LD. Advances on diagnosis of Helicobacter pylori infections. *Crit Rev Microbiol.* 2023;49(6):671-692. <https://doi.org/10.1080/1040841X.2022.2125287>
7. Ricci C, Holton J, Vaira D. Diagnosis of Helicobacter pylori: invasive and non-invasive tests. *Best Pract Res Clin Gastroenterol.* 2007;21(2):299-313. <https://doi.org/10.1016/j.bpg.2006.11.002>
8. Liao G, Gong J, Zhao L, Hou J, Zheng H, Li Y, Wu Y, Chen B. Urease Breath Test and Stool Antigen Test Diagnose Helicobacter Pylori Infection in Patients with Bleeding Peptic Ulcer: a Meta-analysis. *Chin Gen Pract.* 2022;25(3):354.
9. Li ZX, Bronny K, Formichella L, Mejías-Luque R, Burrell T, Macke L, Lang U, Vasapolli R, Hysenaj O, Stallforth I, Vieth M. A multiserological line assay to potentially discriminate current from past Helicobacter pylori infection. *Clinical Microbiology and Infection.* 2024 Jan 1;30(1):114-21. <https://doi.org/10.1016/j.cmi.2023.10.006>
10. Zhang Z, Chen X, Li B, Xia T, Wu X, Wu C. Helicobacter pylori induces urease subunit B-specific CD8+ T cell responses in infected individuals via cytosolic pathway of cross-presentation. *Helicobacter.* 2023 Oct;28(5):e13005. <https://doi.org/10.1111/hel.13005>
11. Fu HW. Helicobacter pylori neutrophil-activating protein: from molecular pathogenesis to clinical applications. *World J Gastroenterol.* 2014;20(18):5294-5306. <https://doi.org/10.3748/wjg.v20.i18.5294>
12. Fu HW, Lai YC. The role of Helicobacter pylori neutrophil-activating protein in the pathogenesis of H. pylori and beyond. *Int J Mol Sci.* 2022;24(1):91. <https://doi.org/10.3390/ijms24010091>
13. Codolo G, Coletta S, D'Elis MM, de Bernard M. HP-NAP of Helicobacter pylori: The Power of Immunomodulation. *Front Immunol.* 2022;13:944139. <https://doi.org/10.3389/fimmu.2022.944139>
14. Mobley H. The role of Helicobacter pylori urease in the pathogenesis of gastritis and peptic ulceration. *Aliment Pharmacol Ther.* 1996;10(Suppl 1):57-64. <https://doi.org/10.1046/j.1365-2036.1996.22164006.x>
15. Donati M, Moreno S, Storni E, Tucci A, Poli L, Mazzoni C, Varoli O, Sambri V, Farencena A, Cevenini R. Detection of serum antibodies to CagA and VacA and of serum neutralizing activity for vacuolating cytotoxin in patients with Helicobacter pylori-induced gastritis. *Clinical Diagnostic Laboratory Immunology.* 1997 Jul;4(4):478-82. <https://doi.org/10.1128/cdli.4.4.478-482.1997>
16. Ekesbo R, Toth E, Fork FT, Held M, Nilsson I, Wadström T, Sjölund K. Chronic Helicobacter pylori infection in a population in southern Sweden analysed by histopathology, immunoblot and ELISA serology. *European journal of gastroenterology & hepatology.* 2006 Jun 1;18(6):589-93. <https://doi.org/10.1097/00042737-200606000-00003>
17. Zalabska E. Does the authentication of the antibodies against Helicobacter pylori have contribution to its diagnosis? *Klin Mikrobiol Infekc Lek.* 2011;17(1):33-37.
18. Reynders MB, Deyi VY, Dahma H, Scheper T, Hanke M, Decolvenaer M, Dediste A. Performance of individual Helicobacter pylori antigens in the immunoblot-based detection of H. pylori infection. *FEMS Immunology & Medical Microbiology.* 2012 Apr 1;64(3):352-63. <https://doi.org/10.1111/j.1574-695X.2011.00920.x>
19. Al Ofairi BA, Saeed MK, Al-Qubaty M, Abdulkareem AM, Al-Jahrani MA. Diagnostic value of IgG antibody and stool antigen tests for chronic Helicobacter pylori infections in Ibb Governorate, Yemen. *Scientific Reports.* 2024 Mar 29;14(1):7536. <https://doi.org/10.1038/s41598-024-58165-w>
20. Kajihara Y, Shimoyama T, Mizuki I. Evaluation of the effects of a proton pump inhibitor on Helicobacter pylori stool antigen testing. *Helicobacter.* 2023;28(3):e12961. <https://doi.org/10.1111/hel.12961>
21. Khorsheed SA. A Comparative Study of Serological and Stool Antigen Tests for Helicobacter pylori Infection Diagnosis. *J Angiother.* 2024;8(4):1-6. <https://doi.org/10.25163/angiotherapy.849578>