Study Protocol

Official Title： The impact of rabeprazole-based triple therapy plus bismuth for first-line *Helicobacter pylori* eradication on the vaginal microecology change: a prospective, randomized controlled trial

NCT number： None

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1. Research Background

Helicobacter pylori (*H. pylori*) is closely associated with chronic gastritis, peptic ulcer, gastric cancer, and gastric MALT lymphoma, and is classified as a Class I carcinogen. Both the 6th National Consensus Conference on Helicobacter pylori in China and the Maastricht Ⅵ Consensus Conference have proposed that "*H. pylori* gastritis is an infectious disease, and *H. pylori*-infected individuals should undergo *H. pylori* eradication unless there are countervailing factors." Currently, guidelines in China recommend bismuth-containing quadruple therapy as the preferred treatment regimen, and selecting an antibiotic regimen with a low resistance rate is conducive to improving the eradication rate. In recent years, multiple studies have shown that Hp eradication therapy has short-term or long-term effects on the gastrointestinal microecology. Gastrointestinal microorganisms and their metabolites affect human health by participating in host endocrine, metabolism, inflammatory responses, and other pathways. The female vaginal microecosystem is part of the human microecosystem, consisting of vaginal microbial flora, endocrine regulatory system, vaginal anatomical structure, and local immune system. The vaginal microbial flora is diverse, with mutual symbiosis

and antagonism, influenced by various internal and external factors, and participates in forming a complex microecosystem. When the vaginal microecological balance is disrupted, changes characterized by abnormal vaginal flora and abnormal vaginal pH value may occur, leading to decreased vaginal resistance to pathogenic microorganisms and secondary infections. To date, there have been no domestic or international research reports on the impact of *H. pylori* eradication therapy on the female vaginal microecology. Therefore, this study intends to conduct a clinical controlled trial among women of childbearing age and menopausal women to explore the effect of *H. pylori* eradication therapy on the vaginal microecology.

1. Research Objectives

1) Clarify the impact of *H. pylori* eradication therapy on the female vaginal microecology;
2) Analyze the safety of the research protocol.

2. Research Design and Methods

(I) Research Method This is a randomized, controlled clinical study. The total research duration of the project is 12 months, starting from the date of ethical approval and completion of clinical registration. The research subjects are patients diagnosed with *H. pylori*-positive chronic gastritis by

gastroscopy due to relevant symptoms. A total of 72 such patients are planned to be enrolled for *H. pylori* eradication therapy, divided into two groups: women of childbearing age and menopausal women, with 36 cases in each group, to explore the impact of *H. pylori* eradication therapy on the vaginal microecology.

(II) Case Selection 1) Inclusion Criteria Randomly select treatment-naive patients with chronic gastritis and positive *H. pylori* confirmed by gastroscopy. According to a computer-generated random number table, 72 patients meeting the inclusion criteria are randomly assigned to the standard *H. pylori* quadruple therapy group (36 cases in each subgroup). All patients participating in the study voluntarily sign the informed consent form. ① Female, aged 18-70 years; ② Positive for *H. pylori*; ③ No use of PPI, H2 receptor antagonists, antibiotics, or bismuth agents within 4 weeks; ④ No prior formal *H. pylori* treatment. 2) Exclusion Criteria ① Use of antibiotics or bismuth agents within 4 weeks before treatment, or use of PPI or H2 receptor antagonists within 2 weeks before treatment; ② History of gastroduodenal surgery; ③ Concurrent presence of other severe diseases such as cardiac, hepatic, or renal insufficiency, malignant tumors, and other serious internal

diseases; ④ Allergy to any component of the study drugs; ⑤ Pregnant or lactating women; ⑥ Participation in other drug studies within 3 months before treatment; ⑦ Inability of the patient to clearly express their main complaints or cooperate with the study. 3) Diagnostic Method for *H. pylori* Infection Before treatment, urea breath test or histological examination of gastric antrum mucosa is performed to assess the *H. pylori* infection status. Patients with a positive result in either test are diagnosed as Hp-infected. (III) Treatment Protocol 1) Treatment Groups: Total enrolled cases: 72 Treatment regimen: Rabeprazole 10mg BID, course of treatment: 14 days Colloidal Bismuth Pectin 220mg BID Amoxicillin 1.0g BID Clarithromycin 0.5g BID Remarks: All drugs used in the study are guaranteed to be from the same manufacturer. - Clarithromycin: Klacid, Abbott Laboratories (Shanghai) Co., Ltd. - Amoxicillin: Nomoling, CSPC Zhongnuo Pharmaceutical (Shijiazhuang) Co., Ltd. - Rabeprazole: Pariet, Eisai Pharmaceutical Co., Ltd. - Colloidal Bismuth Pectin: Bismuth Pectin, Shanxi Ant Biological Pharmaceutical Co., Ltd. 2) Research Flowchart Hp-positive chronic gastritis patients → *H. pylori* quadruple eradication therapy → Postmenopausal women / Women of childbearing age → Vaginal microecology

testing (before treatment, 2 weeks after treatment, 8 weeks after treatment; 6-8 weeks after the end of treatment)

3. Sample Size Calculation

Reference parameters for sample size calculation in this study: δ (non-inferiority margin): -10%, α (significance level) = 0.025 (one-sided), statistical power (1-β) = 0.80 This study is designed with a 1:1 sample ratio between the two groups. Combined with references, SAS 9.2 software (SAS Institute Inc., Cary, NC) is used to calculate that each group requires at least 33 cases. Considering a dropout rate not exceeding 8%, n = 33 / 0.92 ≈ 36. Therefore, at least 72 eligible subjects need to be enrolled.

4. Data Management and Confidentiality

Design and description related to data entry and management: Researchers collect relevant patient information and fill in CRF forms. After data aggregation, a designated person reviews and verifies the data before entering it into the research database. Confidentiality measures for patient data: All records related to the identity of subjects are kept confidential, and such information will not be disclosed to the public beyond the scope permitted by relevant laws and/or regulations.

5. Informed Consent

The informed consent form is signed in writing by the researcher when the patient visits and before planning to participate in the clinical study. The informed consent form and its explanation process shall use language and characters understandable to the subject or their legal representative. During the informed consent process, the researcher shall provide detailed explanations for any content that the subject does not understand or any questions raised about the study, and allow the subject sufficient time to consider whether to participate in the clinical study. After the candidate subject has fully read and understood the content of the informed consent form, if he/she agrees to participate in the clinical study, the subject or their legal representative shall sign and date the informed consent form, and the researcher or their representative who conducts the informed consent process shall also sign and date the form. If neither the subject nor their legal representative is literate, a witness shall be present during the entire informed consent process. After detailed explanation of the informed consent form, the subject or their legal representative gives verbal consent, and the witness signs and dates the form.

6. Adverse Event Reporting

Various adverse events: Timely measures shall be taken for handling, and the events shall be recorded in the case report form. Serious Adverse Events (SAE): Timely measures shall be taken for handling, and the events shall be recorded in the case report form. The researcher decides whether to discontinue or reduce the dosage of the drug, and immediately reports to the Ethics Committee, the drug clinical trial institution, and the sponsor. A report shall be submitted to the national and provincial food and drug regulatory authorities within 24 hours. SAEs must be reported through the "Hospital Intranet Adverse Event and Near-Miss Non-Punitive Reporting System". Specific process: see the figure below. [Figure Description: Occurrence of a serious adverse event (events requiring hospitalization, prolonged hospitalization, disability, impairment of work capacity, life-threatening conditions, death, congenital malformation, etc.) → If no emergency treatment is needed / If emergency treatment is needed, report immediately → Report within 24 hours to the Hospital Medical Affairs Department (Tel: 3880, 3881; for night calls, dial the general duty phone: 660000) → Responsible for proper handling → Report to the State Drug Administration's Safety Supervision Department for Drug Research Supervision (Tel: 010-88363228), Provincial Food and

Drug Administration Registration Department (Tel: 88903275), Ethics Committee, and Sponsor/CRO (Tel: 87783969) → Report to the Adverse Event and Near-Miss Non-Punitive Reporting System via the hospital intranet → Follow up and summarize handling opinions → Report to the hospital's senior management]