Original paper

Eradication success and change in antibiotic resistance after the inclusion of PCR in the approach to *Helicobacter pylori* infection

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Abstract

Introduction: the introduction of PCR testing in the approach to *Helicobacter pylori* (HP) infection could improve diagnosis and HP eradication rates due to its greater diagnostic sensitivity and ability to detect macrolide resistance. The objective of our study was to compare eradication success in relation to 3 different diagnostic strategies based on the microbiological test used. We also analyzed the antibiotic resistance profile of HP in the past decade.

Patients and methods: retrospective analysis of the eradication rate in relation to the implemented microbiological approach (2013-2016: culture; 2017-2019: culture + PCR; 2020-2023: PCR). We calculated the cost-effectiveness ratio (CER) based on the costs of direct diagnostic tests. We analyzed antibiotic resistance in HP isolates between 2013 and 2023.

Results: 288 patients (98 with HP detected by culture, 94 by culture + PCR, 96 by PCR), 228 treated. The eradication rates were 67.5%, 86.2% and 95.8%, respectively, with significant differences between periods 1 and 2 and periods 1 and 3 ($p < 0.001$) but not periods 2 and 3 ($p = 0.087$). Strategy 3 was the most cost-effective (CER 26.74). Macrolide resistance increased progressively (1-16.3%; 2-53.2%; 3-58%). Without PCR, the detected rates would have been 16.3%, 30.8% and 34.3%, respectively. Resistance to other antibiotics remained stable.

Conclusions: the addition of PCR testing resulted in a significant increase in eradication rates. The combination of culture + PCR did not improve these results, and the use of PCR alone was the most cost-effective strategy. Macrolide resistance exceeded 50% in periods when PCR was available.

Key words:
- Antibiotic resistance
- Cost-effectiveness analysis
- *Helicobacter pylori*
- Polymerase chain reaction

Éxito erradicador y cambio en las resistencias antibióticas tras la incorporación de la PCR en el abordaje de la infección por *Helicobacter pylori*

Resumen

Introducción: la incorporación de la reacción en cadena de polimerasa (PCR) en el abordaje de la infección por *Helicobacter pylori* (HP) podría mejorar su diagnóstico y aumentar los porcentajes de erradicación, debido a su mayor sensibilidad diagnóstica y mayor capacidad en la detección de resistencias a macrólidos. El objetivo de este estudio fue comparar el éxito erradicador según 3 estrategias de diagnóstico distintas, en función de la prueba microbiológica utilizada. Se analizaron las resistencias antibióticas de HP en la última década.

Material y métodos: análisis retrospectivo del porcentaje de erradicación según el abordaje microbiológico utilizado (2013-2016: cultivo; 2017-2019: cultivo + PCR; 2020-2023: PCR). Se calculó la *ratio* de análisis de coste-efectividad (CEAR) en función de los costes de las pruebas de diagnóstico directo. Se analizaron las resistencias antibióticas de HP en el periodo 2013-2023.

Resultados: 288 pacientes (98 detectados por cultivo, 94 por cultivo + PCR, 96 por PCR), 228 tratados. Porcentajes de erradicación: 67,5%, 86,2% y 95,8%, respectivamente. Diferencias significativas entre los periodos 1-2 y 1-3 ($p < 0,001$), pero no entre 2-3 ($p = 0,087$). La estrategia 3 fue la más coste-efectiva (CEAR 26,74). Las resistencias a macrólidos aumentaron progresivamente (1-16,3%; 2-53,2%; 3-61,4%). En caso de no disponer de PCR, las resistencias hubieran sido del 16,3%, 30,8% y 34,3%, respectivamente. El resto de las resistencias antibióticas se mantuvieron estables.

Conclusiones: la incorporación de PCR supuso un incremento significativo en los porcentajes de erradicación. La combinación de cultivo + PCR no mejoró estos resultados, siendo la estrategia de solo PCR la más coste-efectiva. Las resistencias a macrólidos sobrepasaron el 50% en los periodos en los que se dispuso de PCR.

Palabras clave:
- Evaluación de coste-efectividad
- *Helicobacter pylori*
- Reacción en cadena de la polimerasa
- Resistencias antibióticas

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INTRODUCTION

Infection by *Helicobacter pylori* (HP) is widely distributed worldwide. The global prevalence of infection is estimated at 40% to 45%, with a slightly decreasing trend in the past decade.[1-3] In children, it is usually asymptomatic, and there is no evidence of an association between infection by HP with chronic abdominal pain compatible with functional disorders.[4-7] In patients with symptoms suggestive of an organic rather than a functional disorder, performance of upper endoscopy with collection of a biopsy sample for direct testing should be considered,[8,9] as this infection can cause complications such as gastric or duodenal ulcers or mucosa-assisted lymphoid tissue (MALT) lymphoma.[10,11]

The infection can be confirmed by means of isolation from culture or positive histopathological identification, in addition to other direct tests, such as polymerase chain reaction (PCR) or the urease test.[12-14] The most recent guidelines of the ESPGHAN/NASPGHAN, published in 2023, consider culture and PCR equally valid for diagnosis, emphasizing the need to target initial therapy based on the results of macrolide susceptibility testing and questioning the usefulness of metronidazole susceptibility testing in culture.[15]

Culture offers a high specificity nearing 100% but a variable sensitivity that ranges from 50 to 90%.[16,17] The advent of molecular techniques has opened new paths and improved diagnostic yield on account of its high sensitivity and specificity (>95%), while offering the opportunity to increase eradication rates thanks to a greater sensitivity in the detection of macrolide resistance.[18-21]

Antibiotic resistance is the leading cause of eradication therapy failure.[9,22] The young age of some patients and difficulties in completing treatment may lead to treatment failure due to abandonment. This, combined with the use of nontargeted "test and treat" strategies or the massive use of antimicrobials in the general population could increase antimicrobial resistance and the frequency of ineffective treatments.[22-26] Therefore, it is essential to conduct antibiotic susceptibility testing before starting eradication therapy to select an appropriate regimen.[13-16] Considering that the costs of culture and PCR are similar,[18] it is important to determine which strategy achieves the best eradication outcomes to ensure rational use of health care resources.

The main objective of our study was to compare eradication success in patients with active HP infection after treatment during 3 different periods, depending on the strategy implemented for diagnosis (culture; culture + PCR or PCR), analyzing the usefulness of direct microbiological diagnostic tests as well as the economic impact of each strategy through a cost-effectiveness study. As a secondary objective, we analyzed local longitudinal trends in HP antibiotic resistance profiles in our area between 2013 and 2023.

MATERIAL AND METHODS

We conducted a cross-sectional, retrospective, observational, and inferential study in pediatric patients aged less than 16 years in whom eradication therapy was initiated after detection of active HP infection between 2013 and 2023. We compared eradication rates in relation to the use of 3 diagnostic strategies, defined according to the ordered microbiological tests (period 1 [2013-2016]: culture; period 2 [2017-2019]: culture + PCR; period 3 [2020-2023]: PCR), and analyzed the cost-effectiveness ratio (CER) in each period.

In all cases, two samples were collected for histopathological examination (one from the antrum and one from the corpus), in addition to another gastric sample for microbiological testing. We defined confirmed infection as positive culture or positive histopathology + positive PCR results. Eradication was defined as a negative result in an indirect test 8 weeks after completion of treatment. In the cost-effectiveness study, we analyzed the costs of the direct microbiological tests (cost of culture: €1 per plate, €24.63 per antimicrobial susceptibility test; cost of PCR: €25) in relation to the number of patients with successful eradication in each period.

We also analyzed local trends in HP antimicrobial resistance between 2013 and 2023 in patients in whom HP was isolated by direct microbiology testing, regardless of whether treatment was initiated.

Patients who received empiric treatment prior to the upper GI endoscopy, patients who do not complete the treatment and patients in whom eradication was not verified with an indirect test were excluded from the analysis of eradication. These patients were, however, included in the analysis of antibiotic resistance.

We summarized qualitative variables as absolute frequencies and percentages, and quantitative variables using the mean and standard deviation. Qualitative and ordinal quantitative variables were compared with the chi-square test or the Fisher exact test, while continuous quantitative variables were compared with the Student t test. The statistical analysis was performed with the software SPSS, version 15.

RESULTS

A total of 288 patients were included, of who 98 were diagnosed by culture (2013-2016), 94 by culture + PCR (2017-2019) and 96 by PCR (2020-2023).

Antibiotherapy was initiated in 228 patients: 90 in period 1 (ESPGHAN 2011 guidelines, sequential therapy), 63 in the period 2 and 75 in period 3 (ESPGHAN 2017 guidelines, treatment based on susceptibility testing results, 14 days' duration). In every case involving strains susceptible to macrolides or metronidazole, the patient received triple therapy with omeprazole, clarithromycin and amoxicillin.

We excluded 7.7% of patients (n = 7) in period 1, 7.9% (n = 5) in period 2 and 3.9% (n = 3) in period 3.

The percentages of successful eradication were 67.5% (n = 56) between 2013 and 2016, 86.2% (n = 50) between 2017 and 2019 and 95.8% (n = 69) between 2020 and 2023 in the per-protocol analysis. We found statistically significant differences in the comparison of periods 1 and 2 (p <0.05), and of periods 1 and 3 (p <0.001), but not when we compared periods 2 and 3 (p = 0.087).

In the cost-effectiveness analysis, PCR testing alone (strategy 3) was the most cost-effective strategy, with a CER of 26.74, compared to the other two options (strategy 1 CER, 37.98; strategy 2 CER, 58.73) (**Table 1**).

With regard to the method used for microbiological detection of HP, 100% of the cases were detected by culture in period 1 and 100% by PCR in period 3. In period 2, 96.8% (91/94) of the cases were detected by PCR and 71.2% (67/94) by culture, with the following distribution: 64 with positive culture and positive PCR, 27 with positive PCR and negative culture, 3 with positive culture and negative PCR, and 13 with negative culture and negative PCR. The sensitivity of culture and PCR in this period was 71.2% and 96.8%, respectively, with a specificity of 100% for both techniques.

With respect to macrolide resistance,16.3% (16/98) of isolates were resistant in the 2013-2016 period. In the 2017-2019 period, the percentage rose to 53.2% (50/94), with resistance detected by PCR in 98% of cases (49/50) and by culture in 58% (29/50). The degree of concordance in the detection of macrolide resistance between PCR and culture was of 55% (27/49). In the 2020-2023 period, 61.4% (59/96) of patients had macrolide resistance. **Table 2** presents the changes in the frequency of macrolide resistance in relation to whether or not PCR was ordered in each period, extrapolating the level of concordance observed between 2017

Table 1. Percentage of successful eradication and cost-effectiveness ratio for each strategy

Strategy	Eradicated/treated cases	Cost (euro)	Cost-effectiveness ratio
Culture	56/83 (67.5%)	2127.29	37.98
Culture + PCR	50/58 (86.2%)	2936.54	58.73
PCR	69/72 (95.8%)	1845.36	26.74

Table 2. Resistance to macrolide with/without PCR by study period			
Strategy	**Macrolide resistance without PCR**	**Macrolide resistance with PCR**	**Statistical significance**
Culture	16.3 % (16/98)	16.3 % (16/98)	$p = 1$
Culture + PCR	30.8% (29/94)	53.2% (50/94)	$p < 0.001$
PCR	34.3% (33/96)	61.4% (59/96)	$p < 0.001$

and 2019. On the other hand, the frequency distribution of resistance to other drugs remained stable (Table 3).

DISCUSSION

In the evaluation of an active HP infection, it is key to identify those patients in whom eradication therapy can achieve significant clinical improvement.[12,13] The high prevalence of functional gastrointestinal disorders in the pediatric population may lead to overdiagnosis and overtreatment of HP infection, since in many cases HP detection is only indicative of colonization and the bacterium is not the cause of the patient's symptoms.[5-7]

Performance of upper endoscopy, when indicated, makes it possible to directly confirm the presence of the bacterium in the stomach, to assess the degree of damage to the gastric mucosa associated with the infection by means of histopathology and to carry out susceptibility testing in order to guide the selection of targeted therapy.[8,9]

Given that the increase in antibiotic resistance is one of the main causes of eradication failure[22-25] and that primary failure may increase the probability of secondary resistance,[9,23] it is essential to set up a detection system that can identify these resistances as reliably as possible.

The introduction of molecular techniques for direct diagnosis opens up new opportunities due to a greater sensitivity and specificity in diagnosis in addition to a greater capacity to detect macrolide resistance.[18-20]

It is worth noting that in period 1, prior to the introduction of molecular diagnostic techniques, the rate of eradication was low, far from the 90% target set by the ESPGHAN. This could be due to the use of culture alone, which has a lower sensitivity for diagnosis and a lower capacity to detect macrolide resistance, as well as the use of treatments that were not guided by susceptibility testing (sequential therapy).

In period 2, when both tests were ordered together, eradication rates increased significantly due to the higher sensitivity of PCR over culture for diagnosis of infection (96.8% vs 71.2%) in addition to a greater capacity to detect macrolide resistance. If PCR had not been available during this period, 28.7% of HP infections (27/94) would not have been diagnosed (corresponding to patients with positive PCR and negative culture). Furthermore, the frequency of macrolide resistance would have been 30.8% (29/94), and macrolide resistance would have not been identified in 22.4% of cases, in which, as a result, eradication may have failed since clarithromycin-based regimens were used in every case in which resistance was not identified.

Table 3. Antibiotic resistance by study period				
Strategy	**Metronidazole**	**Dual resistance (macrolides + metronidazole)**	**Ampicillin**	**Fluoroquinolones**
Culture	7% (7/98)	3% (3/98)	1% (1/98)	7% (7/98)
Culture + PCR	6.3% (6/94)	2% (2/94)	1% (1/94)	7.5% (7/94)
PCR	-	-	-	8.3% (8/96)

In period 3, the rate of eradication increased to 95.8%, with macrolide resistance detected in 61.4% of isolates. Extrapolating the sensitivity data for diagnostic tests during period 2, the frequency of macrolide resistance, without the use of PCR, would have been of 34.3% (33/96), and it is more than likely that the frequency of successful eradication would have also been lower.

The cost-effectiveness analysis showed that the highest eradication rate was achieved with the PCR-only strategy. The culture-only strategy proved to be an inferior option. In period 3, not ordering culture did not result in a decrease in successful eradication compared to period 2, while there was a significant reduction in the use of health care resources.

It should be taken into account that the PCR-only strategy could result in initiation of inappropriate treatment in cases with dual resistance to macrolides and metronidazole.[24-26]. In our area, we found a prevalence of dual resistance of 2.5% between 2013 and 2019,[18] which could translate to prescribing of inappropriate treatment to two patients. However, after the 2023 update of the ESPGHAN/NASPGHAN guidelines, which question the usefulness of metronidazole resistance detected by culture, recommends tailored eradication therapy based on susceptibility testing results for macrolides, which further enhances the utility of PCR.[15]

CONCLUSIONS

We may conclude that the introduction of PCR offers a great opportunity to improve the management and treatment of active HP infection due to its greater diagnostic sensitivity and capacity to detect macrolide resistance. The marked increase in antibiotic resistant strains in the past few decades, together with the risk of resistance going undetected with the exclusive use of culture, may result in the initiation of ineffective treatments, which in turn leads to an increase in secondary resistance and a decrease in eradication rates. The PCR-only strategy proved to be the most cost-effective approach. Table 4 presents the main conclusions of the study.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare in relation to the preparation and publication of this article.

AUTHORSHIP

Author contributions: original concept, data collection and data analysis (MGP and ELOI), contribution of relevant ideas and development of secondary objectives (SMC, CRG, ALV and PGP).

ABBREVIATIONS

CER: cost-effectiveness ratio • **HP:** *Helicobacter pylori* • **PCR:** polymerase chain reaction.

Table 4. Overall results of the study

Estrategia	Pacientes detectados	Pacientes tratados	Pacientes excluidos (estudio de erradicación)	Porcentajes de erradicación	*Ratio* coste-efectividad (CEAR)	Resistencias macrólidos con PCR	Resistencias macrólidos sin PCR
Culture (2013-2016)	98	90	7 (7.7%)	56/83 (67.5%)	37.98	16.3 % (16/98)	16.3 % (16/98)
Culture + PCR (2017-2019)	94	63	5 (7.9%)	50/58 (86.2%)	58.73	53.2% (50/94)	30.8% (29/98)
PCR (2020-2023)	96	75	3 (3.9%)	69/72 (95.8%)	26.74	61.4% (59/96)	34.3% (33/96)

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