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CLO test® revisited

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Abstract

Objectives: To assess the applicability of CLO test® as a rapid bed-side diagnostic test for detection of *H. pylori* organisms.

Design & Setting: Case notes of all patients who had gastroscopies in the medical gastroenterology unit at Withybush Hospital from 14/08/92 to 01/08/94 were reviewed (n=933) and those having PUD not due to NSAIDs were selected for the study. In such patients results of the CLO test® and histology were compared.

Main Outcome Measures: Reasons leading to low positivity of CLO test® in PUD were identified and rectifying measures were worked-out.

Results: CLO test® proved to yield 86% sensitivity and 100% specificity with $P < 0.04$.

Conclusions: CLO test® provided an accurate, cheap, rapid and convenient bed-side diagnostic test to the gastroenterologist to diagnose *H. pylori* to initiate eradication therapy within 24 hours.

Introduction

It is now widely accepted that *H. pylori* is the main cause of duodenal and gastric ulcers. The discovery of the organism, originally called *Campylobacter pyloridis*, and now called *Helicobacter pylori (H. pylori)*[1] is considered one of the most significant advances in gastrointestinal pathology in this century. It is also incriminated in the causation of acute hypochlorohydric gastritis[2], chronic gastritis, duodenitis, duodenal gastric metaplasia, non ulcer dyspepsia, gastric cancer[3,4,5] and primary B cell gastric lymphomas[6]. Various laboratory tests have been designed to diagnose and to assess the response to eradication therapy of *H. pylori.*

The CLO test® *(Campylobacter* Like Organisms test) is most widely used as a rapid bed side diagnostic test, which would enable the Gastro-enterologists to diagnose the presence of *H. pylori* with a high degree of accuracy in a short time to institute eradication therapy in the endoscopy unit itself, in the vast majority of cases. This study has re-evaluated its accuracy in diagnosing the presence of the *H. pylori* in biopsy samples obtained during upper gastrointestinal endoscopy in a group of patients in Prembrokeshire – Wales, U.K., over a 2 year period. The results obtained reconfirmed the high degree of sensitivity and specificity, observed by the other researchers in larger scale studies, and various aspects of shortcommings which could occur in its usage in a busy endoscopy suite is discussed in detail.

Methodology

All patients, who had undergone upper gastrointestinal endoscopy from 14/8/92 to 01/08/94 at Withybush District General Hospital, Haveford West, Prembrokeshire were selected and their endoscopy findings were re-examined. Any patient who had a breach either in the gastric mucosa or/and duodenal mucosa in the form of an either an erosion or an ulcer were included for the study group. The exclusion criteria were;

(1) Those who were on NSAID (Non Steroidal Anti Inflammatory Drugs) at the start.

(2) Those who had carcinoma on histology of the biopsy.

(3) Those who were dead of the time of the study.

Results

Total endoscopy reports re-examined – 933
Total endoscopy reports selected for the study – 53

Sex distribution

Male : Female = 38:15

Age distribution

Years		Numbers
20 - 30	-	2
31 - 40	-	7
41 - 50	-	7
51 - 60	-	6
61 - 70	-	17
71 - 80	-	8
81 - 90	-	6
Total		53

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Endoscopy findings

Gastric ulcers	=	16
Duodenal ulcers (active or healed with scarring)	=	26
Both Gastric & Duodenal ulcers	=	01
Erosive duodenitis and /or gastritis without ulceration	=	10
		53

Performance of CLO test®

Performed - 24
- CLO Positive - 22
- CLO Negative - 2

Unperformed or undocumented - 27 (included 7 HLO negative Duodenal ulcers)

Performance of the biopsy

Yes	-	48
No	-	5

1 - Patient was on warfarin
1 - Patient was in Hepato cellular failure with clotting abnormalities - who had both GU and DUs.
2 - No affordable explanation documented; all had DUs.

Histology

HLO positivity on biopsy - Positive 26; Negative 22

Presence of Chronic Gastritis 38
- HLO (+) - 25
- HLO (-) - 13

Out of 26 HLO (+) biopsies 25 showed the presence of chronic gastritis.

10 reports did not mention the presence of chronic gastritis : 5 patients did not have biopsy reports.

CLO test® and HLO relationship

(1)	Correlated : HLO (+) and CLO (+)	= 18 (70%)
(2)	CLO (+) and HLO (-)	= 3 (2DUs and 1 GU)
(3)	HLO (+) and CLO (-)	= 0
(4)	HLO (-) and CLO (-)	= 2

Comment

(1) The CLO positivity in HLO negative samples is an underestimation as CLO test® was not done in 13 HLO negative biopsies, which included 7 DUs and 6 GUS.

(2) CLO test® was not done on HLO positive biopsies which included 4 DUs and 4 GUs - resulting in a low value for correlation.

	CLO +	CLO -
HLO +	18	0
HLO -	3	2

Fisher exact test P< 0.04: Sensitivity 86% specifying 100%.

Discussion

The diagnosis and detection of *H. pylori* are based on the following methods;

1. Histology which is the "Gold Standard" at present with demonstration of the presence of the organism and associated gastritis. The stains employed are the Haematoxycillin and eosin stain, cresyl-violet, modified Giemsas stain[7] (most widely used) and the Warthin-Starry[8] Silver Stains, but the sensitivity may be still less than 85%[9].
2. Culture of the biopsy specimens which in most important in monitoring the response to therapy, antibiotic resistance and treatment failure.
3. *H. pylori* antibody tests either in serum or saliva which are useful in selecting dysepeptic patients for endoscopy and monitoring of effectiveness of eradication therapy.
4. Biopsy urease test - CLO test® which is cheap, rapid, simple and has a high predictive value. This test is most widely used as a bed side diagnostic test for *H. pylori*, developed by Barry Marshall[10].
5. C^{14} urea breath test which is highly accurate, but expensive and can be used to screen, diagnose and to monitor response to therapy.
6. Polymerase Chain Reaction **(PCR)** in a few centres which will become the "Gold Standard" in future.

H. pylori produces large amounts of urease enzyme[11]. Although urease primarily allows *H. pylori* to utilise urea as a Nitrogen source, the breakdown

of urea also produces, high local concentration of ammonia which enables the organism to tolerate low pH (see reaction below).

$(NH_2)CO +$	$2H_2O +$	$H^+ \rightarrow$	$2NH_4^+ +$	HCO_3
Urea	Water	Hydrogen Ion	Ammonia Ion	Bicarbonate Ion

Tests for gastric urease are specific for *H. pylori*, as mammalian cells do not produce urease and except for *H. pylori*, the stomach is usually sterile.

The CLO test® is a sealed plastic slide with a well containing an agar gel which contains, urea, pherol red (a pH indicator), buffers and bacteriostatic agents. If the urease enzyme of *H. pylori* is present in the inserted tissue sample, the degraded urea causes a high pH and the colour of the gel turns from yellow to a bright magenta colour. The kits should be refrigerated at 2-8° C, and have shelf life of 18 months.

When selecting patients for endoscopy to perform CLO test®, the patients should not have taken antibiotics, bismuth salts or proton pump inhibitors, at least 3 weeks prior to endoscopy, as suppression of the organism can occur. Re-growth may be patchy, and can give rise to false negative results. In the study, review of the case notes of the selected patients, revealed that much attention had not been paid to document the prescription of antibiotics or proton pump inhibitors in the preceding 3 weeks, in almost all cases, except if they were included in the list of medication on admission. If this were accurately documented, it would have helped to explain some of the HLO and CLO test® descrepancies. Two patients in the study, having CLO test® (-) DUs, were on a proton pump inhibitor - omeprazole.

The functional kit should have a yellow colour at the start, and should be warmed at 30-40° C immediately before use, which would help speeding up the reaction. An adequate biopsy from an appropriate site is placed inside the well, and the kit is then placed in a warm place (30-40° C) for the next 3 hours, after which time, kept at room temperature (20° C). The CLO test® is examined at intervals of 20 minutes, 1 hour, 3 hours and 24 hours, respectively, after insertion of the biopsy. A positive result is indicated either by an expanding pink zone around the biopsy or a deep orange colour which will turn later into magenta pink. The latter occurs when there is a small number of organisms. A negative result will remain yellow at 24 hours.

The CLO test® will diagnose 70% of *H. pylori* infections is the endoscopy room (20 minutes), with no false positives at that time[12]. By 1 hour 85%, and by 3 hours 90% of the positive cases will be detected. Between 3 hours and 24 hours, another 5% of the patients will be detected. In the largest U.S. study reported to date by Dye et al[13] took antral biopsies, one each for histology (Giemsa's Stain) and CLO test® from 122 consecutive routine endoscopy patients at the University of Virginia. 82 patients also had specimen cultured.

CLO tests® were read by the endoscopy nurse. The results obtained are shown in the table below. In 80% of the patients, the test become positive in 1 hour. Unfortunately this information had not been documented in any of the patients studied in our series, to compare the results.

	True +	True -	False +	False -
CLO test (n=121)	46	72	2	1
Histology (n=122)	43	74	-	5
Culture (n=82)	23	49	-	10

	CLO test®	Histology	Culture
Sensitivity	98%	91%	70%
Specificity	97%	100%	100%

Similar results have been obtained by Schenell et al in Missouri[14]. In our own series, the sensitivity of the CLO test® was around 80% and specificity reached 100% respectively and the following would have influenced the results Viz.

(1) Biopsies were not done in 5 patients and therefore the HLO and CLO test® results were unknown thus changing the nett result.

(2) CLO test® was either not performed or not documented in 27 cases.

(3) Inadequacy of the biopsy sample numbers. The recommended ideal numbers are 2 samples from the anturm and 2 samples from the body respectively, as sometimes, the growth may be patchy, including the body of the stomach.

(4) 2 patients were on proton pump inhibitors at the time of the test, which could have lead to a false negative result.

(5) Antibiotic or proton pump inhibitor treatment in the preceeding 3 weeks were not inquired into, prior to endoscopy except when they were included in the list of medication on admission.

(6) On retrospective examination of the CLO test® positive, HLO negative biopsy specimens by the pathologists at Withybush District General Hospital, it was able to identify the presence of a few HLO in 2 specimens which were previously reported as HLO negative, but were CLO positive. Had this result been available at the time of the original study, results would have been different.

The CLO test®, as any other test has its inherent draw backs. A false negative result can occur when the numbers of H. pylori are very small and has a patchy distribution as, in 1-5% of patients it is in the body, but not in the antrum. Similarly areas of intestinal metaplasia do not harbour the organism, and will fail to reveal if biopsied. Prior use of antibiotics, bismuth salts and proton pump inhibitors, affect adversely on the positivity. False positive results are rare and the 2 cases reported by Dye et al[15] were due to faulty kits. Theoretically, this could occur in condition of hypochlorhydria[16], for example, patients having pernicious anaemia, previous gastric surgery, or who have recently taken antacid, or large doses of H_2 receptors antagonists which would allow the commensal organisms such as *Klebsiella* and *Proteus* Spp, to grow in the stomach producing urease[17,18]. False positive reactions due to bacteria other than *H. pylori,* will usually not react before 3 hours, as these organisms produce much less urease than *H. pylori.* False positives can also occur if read after 24 hours.

Clinical implications

This study re-confirms the usefulness of the CLO test® as a rapid, easy to perform, sensitive, bedside diagnostic test, available to the Gastroenterologist to detect presence of *H.pylori* in biopsy samples and to institute therapy where appropriate, before the results of histology or culture, within 24 hours. In a vast majority, within 3 hours. This study also highlights the various aspects, which should be looked into, in order to get an accurate result, when selecting patients for endoscopy and performing the CLO test® to detect *H.pylori.*

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