

Role of Chronic Infection and Inflammation in the Gastrointestinal Tract in the Etiology and Pathogenesis of Idiopathic Parkinsonism

Part 2: Response of Facets of Clinical Idiopathic Parkinsonism to *Helicobacter pylori* Eradication. A randomized, Double-blind, Placebo-controlled Efficacy Study

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ABSTRACT

Background. Links between etiology/pathogenesis of neuropsychiatric disease and infection are increasingly recognized.

Aim. Proof-of-principle that infection contributes to idiopathic parkinsonism.

Methods. Randomized, double-blind, placebo-controlled efficacy study of proven *Helicobacter pylori* eradication on the time course of facets of parkinsonism. Intervention was 1 week's triple eradication therapy/placebos. Routine deblinding at 1 year (those still infected received open-active), with follow-up to 5 years post-eradication. Primary outcome was mean stride length at free-walking speed, sample size 56 for a difference, active vs. placebo, of 3/4 (between-subject standard deviation). Recruitment of subjects with idiopathic parkinsonism and *H. pylori* infection was stopped at 31, because of marked deterioration with eradication failure. Interim analysis was made in the 20 who had reached deblinding, seven of whom were receiving antiparkinsonian medication (long- $t_{1/2}$, evenly spaced) which remained unchanged.

Results. Improvement in stride-length, on active (n = 9) vs. placebo (11), exceeded size of effect on which

the sample size was calculated when analyzed on intention-to-treat basis ($p = .02$), and on protocol analysis of six weekly assessments, including ($p = .02$) and excluding ($p = .05$) those on antiparkinsonian medication. Active eradication (blind or open) failed in 4/20, in whom B-lymphocyte count was lower. Their mean time course was: for stride-length, -243 (95% CI -427, -60) vs. 45 (-10, 100) mm/year in the remainder ($p = .001$); for the ratio, torque to extend to flex relaxed arm, 349 (146, 718) vs. 58 (27, 96)%/year ($p < .001$); and for independently rated, visual-analog scale of stance-walk videos (worst-best per individual \equiv 0-100 mm), -64 vs. -3 mm from anterior and -50 vs. 11 lateral ($p = .004$ and $.02$).

Conclusions. Interim analysis points to a direct or surrogate (not necessarily unique) role of a particular infection in the pathogenesis of parkinsonism. With eradication failure, bolus release of antigen from killed bacteria could aggravate an effect of ongoing infection.

Keywords. *Helicobacter pylori*, efficacy of eradication, placebo-controlled, idiopathic parkinsonism, objective measures facets, lymphopenia.

We seek proof-of-principle for a pathogenic role of infection in idiopathic parkinsonism by studying the effects of *Helicobacter pylori*

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eradication on measures of its facets. The term idiopathic parkinsonism [1] is employed to describe a clinical syndrome of unknown etiology, while putting certain parkinsonian states and wider clinical entities off-limits. Its manifestation is taken to be the nett effect of "doses" of genetic predisposition and environmental risk factors [2].

Why target *Helicobacter*? Two decades before the description of *H. pylori*-induced gastritis, an excess of previously diagnosed peptic ulcer in parkinsonism was documented [3]. Concomitant ulcer was rare. Atschuler speculated on a causal link [4]. We substantiated the fit and proposed that acquired immunosuppression [5], plus increased circulating interleukin-6 [6], result in autoimmune tissue damage, manifest as parkinsonism. Most *Helicobacter* infections are transmitted, within a closely shared environment, from parent or sibling to infant [7]. Similarly, the younger the child when the parent develops parkinsonism, the greater the risk thereof to that child [8]. Proband with idiopathic parkinsonism, aged ≤ 72.5 years, are twice [9], and their siblings thrice [10], as likely as controls to be seropositive for specific *H. pylori* anti-urease-IgG antibody (directed against high molecular weight, cell-bound enzyme, and measured by enzyme-linked immunosorbent assay [ELISA]). Obliteration of the birth-cohort effect in antibody titer in parkinsonism is compatible with causality (as in peptic ulcer/gastric carcinoma), elimination of infection, and/or progressive immunocompromise [9]. By age 80, the predicted probability of being labeled parkinsonian is five times greater in the presence of antibodies against *H. pylori* cytotoxin-associated-gene-A product (CagA), but absence of vacuolating-toxin (VacA) and urease-B antibodies, than in the absence of all three [11]. Therefore, previous, rather than current, infection appears characteristic. Spontaneous eradication, or incidental (by antimicrobial/acid-suppressant/antiparkinsonian medicines), might explain "quieter" parkinsonism, persistent infection aggressive parkinsonism with premature death [5].

In pragmatic hypothesis testing, sensitive, specific, continuous objective measures are economical for sample size. In parkinsonism, subjective scores hold sway, but are blunt instruments [12]. In contrast, mean stride-length at free-walking speed usefully defines treatment effect [13]. When corrected for relevant demographic/anthropometric characteristics, it discriminates well between those with and without diagnosed parkinsonism [14]. In the efficacy study presented, cardinal outcome criteria are 1, "hypokinesia" as measured by stride length, measurements of 2, arm "rigidity" [15,16] and of 3, "postural abnormality" by standing body sway and ambulatory foot-separation [17,18], and 4, amplitude/frequency of characteristic "tremor" under set conditions [15]. Reaction time tests document

associated psychomotor and psychometric disability [19]. Applying a visual-analog scale gives continuous numerical data to a video archive of stance and walking.

Methods

Subjects

The study was approved by local ethics committees and written informed consent obtained from all participants. Figure 1 shows the profile. Subjects with "clinically definite" [1] idiopathic parkinsonism were eligible for screening of *Helicobacter* and immune status. *Helicobacter* status was defined by [^{13}C] urea breath test (UBT) (INFAI, Kestrel Healthcare Ltd, Basingstoke, UK), ELISA value (EV) for serum anti-urease-IgG antibody and immunoblot profile of serum antibodies against a range of *H. pylori* antigens (*Immunological Methods*). Positive *H. pylori* status was confirmed by esophago-gastro-duodenoscopy. Accompanying spouses/partners were offered screening.

At endoscopy, individuals could opt for "light" sedation (intravenous midazolam: maximum dose 4 mg). Infection was proven by 1, gastritis with associated *Helicobacter*-like organisms on microscopy (any of six biopsies: anterior and posterior antral, incisural, anterior and posterior corporal and cardiac); and/or 2, culture of *H. pylori* (either of two biopsies: antral and corporal, couriered to the *Helicobacter* Reference Laboratory); or, if culture negative 3, detection of *Helicobacter* DNA.

Table 1 gives efficacy study inclusion/exclusion criteria. Subjects receiving no antiparkinsonian medication were preferred. Any such medication had to be evenly spaced with long $t_{1/2}$, and remain constant throughout.

Efficacy Study Protocol

This was a randomized, double-blind, placebo-controlled, parallel-group study of the effect of *H. pylori* eradication on facets of idiopathic parkinsonism. One group received triple therapy, the other matched-placebo capsules/tablets. Active treatment was 20 mg omeprazole, 500 mg clarithromycin, and 1 g amoxicillin, 12-hourly for 1 week. Twelve-hourly metronidazole 400 mg and/or 6-hourly tetracycline 500 mg (or respective placebo[s]) was substituted where there was in vitro insensitivity and/or suspected intolerance. Compliance was checked by telephone.

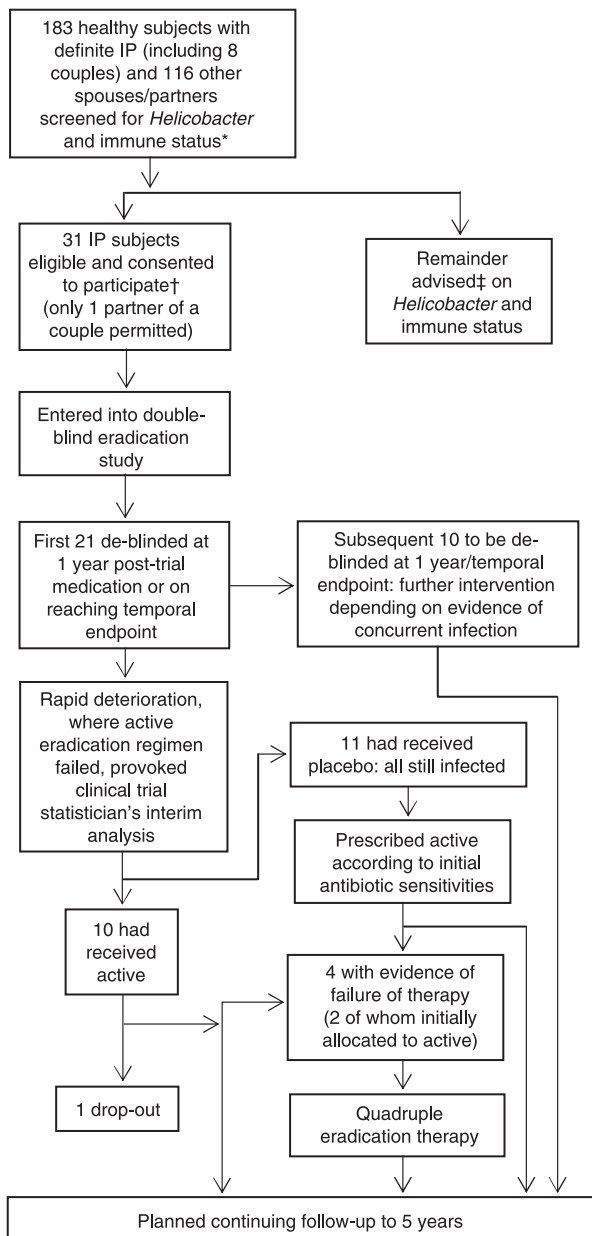


Figure 1 Study profile in probands with idiopathic parkinsonism (IP) and their spouses/partners. *Of 299 subjects screened, 40.1% were immunoblot positive, 7.3% equivocal. Only 21.5% were EV and 19.1% UBT positive. †Current infection proven by culture in 29/31 subjects. Remaining two positive on PCR. *H. pylori*-like organisms on histology in 30, the exception being culture positive. Twenty-nine had been positive on all three screening tests, one UBT negative, another with equivocal EV. ‡With reference to further investigation and treatment.

Two baseline assessments, at a set time of day, preceded randomization. After treatment, assessments were approximately 6-weekly. Deblinding was scheduled for 54 weeks, or sooner if 1, current lifestyle threatened by disease progression

(“temporal end-point”); 2, other exit criteria met (development of other medical conditions needing specialist attention, falls with serious injury/fracture, prescription of new, or modification to, antiparkinsonian medication); or 3, at request of subject. Judgments, with subject safety paramount, were made blind-to-treatment by the physician responsible for day-to-day management, in consultation with another.

At deblinding following an active regimen, UBT and repeat of the biopsy procedure are offered. Those who received placebo and remain UBT positive are offered the corresponding active regimen, with six weekly follow-ups to 1 year. (Endoscopic confirmation of apparent spontaneous eradication would be offered.) Following open-active, UBT is offered for 6 weeks, with, if negative, a delay to endoscopy of at least 4 months. Where there is evidence that a blind- or open-active regimen failed, quadruple therapy is offered, based on new antimicrobial sensitivities/inferences from polymerase chain reaction (PCR) assays. Where there is no evidence of failure, follow-up is reduced to 12-weekly for 1 year, and 24-weekly for the next three years.

Clinical Measurements

For gait analysis [13], rested subjects walked unaided for 18 m in a 1.85 m wide corridor, “at your own speed”. A second walk followed 5 minutes rest. Mean stride-length, free-walking speed, peak-limb-velocity and mean foot-separation at mid-swing for “steady state” gait were analyzed [21].

To measure rigidity [15], the supported forearm was moved horizontally, at a controlled velocity, through a 40° arc (115–155°) about the elbow. The side judged more rigid at the initial visit was used. Swing duration was 1.3 seconds, the pause between varying (1–3 seconds) to reduce the effect of anticipation. One minute’s acclimatization preceded 2.5 minutes recording. A computer interface unit measured the torque required for passive displacement against position (sampling interval 25 milliseconds). Mean torque for each swing in extension, and in flexion, was calculated, and the ratio of the grand means taken. (Flexor rigidity > extensor is characteristic of parkinsonism.)

Total angular displacement (sway) in the sagittal plane was measured [15]: subjects stood at ease, with eyes open for 1 minute, then closed for three. Anatomical posture was gauged by height

Table 1 Efficacy study inclusion and exclusion criteria**Inclusion**

Independently living subjects with clinically definite^a idiopathic parkinsonism and *H. pylori* infection, proven on endoscopic gastric biopsy. Any antiparkinsonian medication^b had to be at steady-state, and evenly spaced, with reference to (a long) $t_{1/2}$, to avoid iatrogenic fluctuations in performance.

Exclusion

1. Secondary parkinsonism, "parkinsonism-plus" syndromes and other wider clinical entities [1,2]
2. Use of levodopa
3. Other progressive or resolving disorders affecting physical ability or performance, or sufficient underlying incapacity to prevent assessments
4. Clinical depression, dementia or other mental illness, or a score $\leq 8/16$ on the Modified Tooting Bec Questionnaire [15] and/or $< 24/30$ on Mini-mental Score [20]
5. Other specific neurological condition
6. Cardiovascular/respiratory symptoms during normal activities
7. UK MRC Muscle Strength Score $< 4/5$
8. Concurrent therapy with drugs which might be antidopaminergic, or with hypnotics or sedatives
9. Recent change in life situation
10. Serious pathology, such as ulcer or neoplasm, at endoscopy^c

^aAny combination of three of the features: resting tremor, rigidity, bradykinesia, or impairment of postural reflexes. Alternatively sufficient were two of these features, with one of the first three displaying asymmetry [1].

^bCall for volunteers emphasized the need for those who have not yet been exposed to antiparkinsonian medication, and set-out conditions if receiving it.

^cEsophageal or gastro-duodenal lesions biopsied in line with standard practice.

and forward displacement of occiput [18]: buttocks and heels against a wall, but otherwise standing relaxed. Body weight was recorded.

A fixed camera filmed a plan view of hands, resting semiprone on the table in front of the relaxed seated subject. It recorded any tremor over 1 minute before and during repeating-back, in reverse order, series of spoken random numbers [15].

Reaction time was measured as that taken to lift left or right index finger from its touch-sensitive support [19]. Two seconds before the imperative, an alerting signal did, or did not, warn whether left or right was to be lifted. The ratio, unwarned/warned, was taken ("cognitive efficiency").

An independent assessor, blind to date and treatment, analyzed an archive of anterior and lateral videos of standing (15 seconds) followed by walking (over 12.5 and 6.5 m, respectively). Lateral perspective was from same side as used for rigidity. He applied a visual-analog scale for each perspective, 0 mm representing an individual's worst walk, 100 his/her best. Another reviewed a sample (5 individuals, 44 occasions). Concordance [22] between assessors was encouraging (correlation coefficient 0.77 [95% CI 0.64, 0.89] for anterior perspective, 0.85 [0.75, 0.94] for lateral). There was no systematic bias (mean between-assessor difference in rating -3 [$-13, 7$] and 8 mm [$-1, 18$], respectively).

Immunological, Microbiological, and Histological Methods

Serum was stored at -20 °C. For antiurease-IgG antibody (SIA417A, Delta Biologicals, Rome),

between-assay coefficients of variation, for the samples assayed in singlicate, were 5.8 and 8.1% at an ELISA value of 1.7 and 3.2. An EV > 2.2 is the recommended cut-point of seropositivity, from equivocal/seronegative ($2.2-1.8/ < 1.8$). Presence/absence of defined antibody bands on Western Blotting was scored according to the manufacturer's system (RIDA *Helicobacter* Blot IgG, Quadratch Diagnostics Ltd, Epsom, UK). A score > 12 is regarded as positive, 11–12 equivocal and < 11 negative. Each assay kit contains a negative and a positive quality control. Blots and the developed control (k) strip, supplied with each kit, were scanned and magnified, to maximize precision of band detection.

Standard reference laboratory operating procedures [23–26] for *H. pylori* culture and testing in vitro susceptibility to clarithromycin, amoxicillin, metronidazole, and tetracycline were followed. Culture-negative biopsies were tested for *H. pylori* by PCR [27] and, where positive, molecular inferences on antibiotic sensitivities made (Table 2 footnote).

Biopsies, oriented from proximal to distal on a cellulose acetate strip, were fixed in 10% buffered formalin, and processed to paraffin-blocks. Sections were stained with H&E and organisms sought using cresyl-fast violet. One pathologist examined and classified (Updated Sydney System [28]) all.

Statistical Methods

The sample-size calculation was based on the primary outcome criterion, mean stride-length.

Characteristics	Mean (data interval)	
	Placebo (n = 11)	Active (n = 9 ^a)
Demographic/anthropometric		
Age year	62 (41, 83)	59 (38, 80)
Gender	8M, 3F	2M, 7F
Height m	1.71 (1.54, 1.86)	1.67 (1.43, 1.90)
Weight kg	77.6 (58.0, 97.2)	76.0 (45.3, 106.6)
Mini-mental score (maximum 30)	28 (27, 29) ^c	29 (27, 30) ^c
Modified Tooting Bec score (16) ^b	15 (14.5, 15.5) ^c	15.5 (15.5, 16) ^c
Beck's hopelessness score (20)	3 (1, 5) ^c	4 (2, 7) ^c
Brady/hypokinesia		
Mean stride length mm	1229 (835, 1623)	1209 (811, 1607)
Free walking speed m/s	1.18 (0.7, 1.66)	1.15 (0.63, 1.67)
Rigidity		
Mean torque to extend forearm mNm	406 (192, 863)	410 (178, 943)
Mean torque to flex mNm	266 (104, 677)	284 (148, 546)
Postural abnormality		
Mean body sway, eyes opened °	5.3 (1.8, 16.1)	5.6 (2.4, 12.4)
Mean body sway, eyes closed °	9.0 (2.6, 31.5)	8.5 (2.6, 27.9)
Mean foot-separation mm	202 (156, 248)	190 (142, 238)
Psychomotor and psychometric measures		
Mean unwarned reaction time ms	645 (440, 946)	592 (362, 968)
Mean warned reaction time ms	381 (234, 650)	335 (201, 558)
Histopathology and microbiology on gastric biopsies		
<i>Helicobacter</i> -like organisms on histology ^d	11	9
Culture +ve	11	8 ^e
Metronidazole resistance, antral biopsy ^f	3 (1)	3 (2)
Metronidazole resistance, body ^f	3 (1)	2 (2)
Immunoblot +ve for CagA antibody	8	8

^aExcluding drop-out immediately post-trial medication (nonmedical reason).

^bStatistically significant ($p = .03$) difference between treatment designations, by play of chance, for this characteristic only.

^cMedian (interquartile range).

^dPangastritis (n = 7), antral predominant (12), corpus predominant (1). Atrophy (7), intestinal metaplasia (4): both moderate in one subject, minimal/mild in remainder.

^eIn remaining subject, PCR for *H. pylori* (*vacA*) positive on both biopsies, clarithromycin mutation assay showing wild (sensitive) type.

^fNumber with metronidazole resistance (intermediate susceptibility). All cultures sensitive to clarithromycin, amoxicillin and tetracycline. Second line antimicrobials prescribed in two subjects (active group) because of history of intolerance: Rx clarithromycin with metronidazole in one, with tetracycline in other.

For a type I error of 0.05 and a type II of 0.2, 56 subjects (28 in each group) would be required to show a difference, at 1 year, between active and placebo treatments of three quarters of the between-subject standard deviation (SD). Interim analysis was planned at completion of the double-blind phase.

While detailing drop-outs and outcome of treatment failure are integral to any efficacy study, in the double-blind protocol analysis, treatment refers to "successful" active (no evidence of failure) or placebo. A two-level generalized linear mixed model [29] was used to analyze repeated measures of primary, other cardinal and associated-disability outcome criteria, and those on a visual-analog scale. Fixed effects in the model were: nature of treatment; time since treatment; any background antiparkinsonian medication; their interactions (between: treatment and time,

background-medication status and time, treatment and background-medication status); mean baseline measurement; and, where relevant, demographic/anthropometric characteristics. Random effects related to the within-subject residual and, at the subject-level, to intercept and slope, which were assumed to have a bivariate normal distribution. Given a random effect of subject, there are sufficient remaining degrees of freedom not to occasion removal of model terms. Thus, main effects were kept in the model regardless of whether they reached conventional levels of statistical significance. Models were fitted via the generalized linear latent and mixed model (GLLAMM) command [30] within STATA 8 [31], using adaptive quadrature. Efficacy of eradication was assessed via the interaction between nature of and time since treatment. Estimated annual change in an outcome was

Table 2 Prerandomization characteristics of efficacy study subjects

obtained directly, or by linear combination of model coefficients, according to nature of treatment.

In the case of eradication failure, nature of treatment would refer to failure or success of all active therapy, provided there was no interaction between blind and open.

Variables exhibiting a positively-skewed distribution were transformed (natural logarithmic) to approximate normality. For visual-analog assessments, an arcsine transformation ensured that ceiling and floor constraints were not violated.

Results

Screening: Helicobacter and Immune Status

On screening (Fig. 1), 40% were immunoblot-positive, twice as many as EV- or UBT-positive. There was no significant difference between probands and spouses/partners in *Helicobacter* status by any of these tests. Figure 2A shows the downward shift in circulating lymphocyte count in relation to the lower reference range limit in both probands and spouses/partners. Of probands, 36.5% fell below, but only 14.9% of spouses/partners did. Considering only the couples, 15.3% (95% CI 5.8, 24.8) more probands than spouses/partners had lymphopenia ($p = .001$). Anti-parkinsonian medication (70.5%) did not affect the probands' lymphocyte count. There was no within-couple concordance, in either *Helicobacter* status or lymphocyte count, beyond what could be explained by chance [22,32].

Figure 2B shows that a positive immunoblot is associated with a higher lymphocyte count ($p = .03$, gender and age adjusted, and irrespective of subject group). A trend ($p < .06$) of similar magnitude is seen with an equivocal immunoblot. Neither EV nor UBT was predictive of cell count.

Efficacy Study: Decision to Halt Recruitment

The physician responsible for day-to-day management alerted the clinical trial statistician to marked deterioration where blind- or open-active *Helicobacter* eradication therapy failed. At this time, 31 subjects had been randomized, including one drop-out with no data after blinded treatment (Fig. 1).

An intention-to-treat analysis was performed, on mean stride-length at the final blind evaluation, in the 20 subjects who had reached debinding. Multivariable linear regression analysis was used, adjusting for mean baseline stride-length, relevant demographic/anthropometric covariates and time to debinding. (Table 2 shows that randomization resulted in two groups with no major imbalance in baseline clinical measurements or pretreatment histopathology/microbiology.) The intention-to-treat hypothesis test gave a p value of .02, after adjustment for baseline stride-length ($p < .001$) and age (< 0.02), neither height and gender [14] nor time to debinding contributing to variance explained. Stride-length was 67 mm (95% CI 12, 121) greater after active treatment (nine subjects) than after placebo (11 subjects), at the final blind evaluation. The magnitude of effect was 1.18 (SD), whereas the study was designed to detect 0.75 (SD) with a sample size of 56.

Steering and ethical committees were informed of safety and efficacy issues, recruitment halted at 31 subjects, and interim analysis of repeated measures expedited in the 20 debinded.

Analysis of Repeated Measures

Table 3 gives the detailed analysis of time-trends in primary, other cardinal and associated-disability outcomes, and visual-analog rating of stance-walk videos. This is categorized according to 1,

Figure 2 Lymphocyte count in idiopathic parkinsonism and spouses/partners of probands in relation to *H. pylori* status. (A) Circulating lymphocyte count in 104 probands (P) and 67 spouses/partners (Ps) compared with lower reference range limit (-----). (B) Mean (95% CI) lymphocyte count in all 171 subjects (adjusted to age 60 years and as if all male) in relation to *H. pylori* immunoblot status.

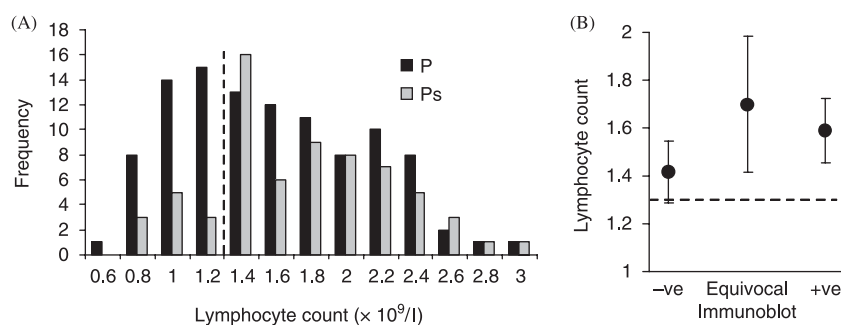


Table 3 Safety and efficacy of *Helicobacter* eradication therapy in idiopathic parkinsonism. Within-subject time-trends in objective and visual-analog outcome measures

Outcome variable	Estimated change/year ^a mean (95% CI)			Estimated change/year mean (95% CI)			Estimated change/ year ^b mean (95% CI)		
	Evidence failed eradication ^c	Remainder	<i>p</i> value	Remainder Background medicationanti-IP		<i>p</i> value	Placebo	Active	<i>p</i> value
	22 assessments (n = 4)	110 assessments (n = 16)		Absent 91 assessments (n = 13)	Present 19 assessments (n = 3)		55 assessments (n = 11)	49 assessments (n = 7)	
Hypokinesia									
Mean stride length mm	-243 (-427, -60)	45 (-10, 100)	0.001	40 (-17, 96)	244 (100, 389)	0.008	-10 (-54, 34)	59 (18, 100)	0.02 ^d
Rigidity percentage									
Torque: extension/flexion	349 (146, 718)	58 (27, 96)	< 0.001	61 (29, 100)	-26 (-56, 26)	0.006	66 (15, 141)	19 (-14, 65)	0.2
Mean torque to extend	47 (-51, 341)	9.3 (-23, 54)	0.6	15 (-18, 60)	19 (-44, 154)	0.9	34 (6, 69)	70 (37, 112)	0.1
Mean torque to flex	-49 (-77, 13)	-21 (-39, 2)	0.2	-21 (-40, 4)	39 (-31, 177)	0.1	-18 (-41, 15)	44 (10, 87)	0.008
Postural abnormality									
Body sway percentage ^e	-19 (-44, 18)	-3 (-13, 8)	0.3	-2 (-13, 11)	15 (-15, 54)	0.3	-6 (-10, -2)	3 (-1, 7)	0.006
Mean foot separation mm	-6 (-39, 27)	-15 (-25, -4)	0.6	-17 (-30, -5)	-25 (-52, 1)	0.6	1 (-19, 21)	-1 (-20, 18)	0.9
Psychomotor & psychometric measures percentage									
Mean reaction time	-47 (-67, -15)	-22 (-33, -10)	0.09	-22 (-44, -8)	16 (-21, 7)	0.06	-11 (-31, 14)	-20 (-36, 0)	0.5
Cognitive efficiency	3 (-31, 55)	0 (-9, 10)	0.9	-1 (-10, 9)	4 (-25, 43)	0.8	-5 (-20, 12)	-2 (-17, 15)	0.8
Visual-analog rating of stance-walk videos mm^f									
Lateral perspective	-50 (<i>p</i> = .1)	11 (<i>p</i> = .3)	0.02	14 (<i>p</i> = .3)	16 (<i>p</i> = .3)	0.7	1 (<i>p</i> = 1.0)	27 (<i>p</i> = .1)	0.3
Anterior perspective	-64 (<i>p</i> = .003)	-3 (<i>p</i> = .6)	0.004	-3 (<i>p</i> = .5)	31 (<i>p</i> = .06)	0.04	11 (<i>p</i> = .5)	-2 (<i>p</i> = .9)	0.5

^aMedian duration of follow-up in successes 478 days (interquartile range 345–665), in failures 305 (96–616).

^bMedian follow-up 371 (329–409) days following active treatment and 126 (77–387) following placebo. Temporal endpoint reached in 6/11 subjects allocated placebo, but only 1/9 allocated active. One other in each group requested early de-blinding (intolerance of uncertainty).

^cCriterion: breath test (n = 1), histology and culture (1), molecular detection only (2). UBT available in all 20 following any active treatment. Sixteen out of twenty had a repeat endoscopy at a median of 356 (interquartile range 224, 370) days after blind- or open-active eradication therapy. Classification and grading of gastritis improved (even in those with evidence of failed eradication), or returned to normal.

^dDifference between treatments remained significant at *p* ≤ .05 after excluding those on background antiparkinsonian medication, size of effect similar to above [-3 (-50, 45) mm/year for placebo (n = 7) and 58 (16, 101) mm/year for active (n = 6)].

^eNo interaction between treatment and condition (eyes open or closed).

^fPoint estimate (significance) of time-trend given, since interval estimates not tractable to back-transformation (see Statistical Methods).

failure or success of eradication, with subdivision of successes by presence/absence of background antiparkinsonian medication, and 2, double-blind protocol analysis. Footnotes a and b give numbers reaching a temporal endpoint, as well as duration of follow-up. All 20 deblinded subjects continued as per schedule, contributing to the total durations of follow-up given for failed or successful eradication. Any antiparkinsonian medication was constant throughout this period.

Tremor analysis is reserved for the complete cohort of 30 subjects, since only 8/20 had tremor under video conditions.

Adverse Outcome of Failed Eradication

The clinical impression of marked deterioration in parkinsonism with eradication failure was consolidated by three aspects of data analysis (Fig. 3).

Mean stride-length declined by 288 mm/year, on average, with eradication failure relative to success. There were concomitant relative declines in other distance/time gait indices: free-walking speed by 0.26 m/second/year ($p < .001$) and peak-limb-velocity by 0.4 m/second/year ($p = .08$). All failures were receiving background antiparkinsonian medicine. So were three of the successes, but their stride-length improved by 244 mm/year (Table 3, $p = .001$).

Ratio of torque to extend to flex the forearm, increased by 291% per year with eradication failure, relative to success. The modest increase in ratio with successful eradication (58% per year) appeared blocked by background medication (Table 3, $p = .006$). Any such effect was overwhelmed where eradication failed.

Ratings of both anterior and lateral stance-walk videos deteriorated by 61% per year with failure of eradication, relative to success. Here also, the benefit of success was greater (Table 3,

34% on anterior videos, $p = .04$) in those receiving background medication.

Immune status was a determinant of eradication failure. Those in whom eradication failed were not characterized by red cell, total white cell, neutrophil, lymphocyte or platelet counts, nor by T-helper (CD4+) or natural killer (CD16+56+) lymphocyte-subset counts. However, their mean cytotoxic T cell (CD8+) count, 328 (95% CI 161, 494)/ μl , was below the reference range (391–793), and lower by 244 (–28, 516) than in successes ($p = .08$). Their mean B-cell (CD19+) count was just within normal range, but lower by 130 (15, 246)/ μl than in successes ($p = .03$).

Double-blind Protocol Analysis

More reached a temporal endpoint, with consequent early deblinding, after placebo than after active (Table 3 footnote^b). Moreover, the time-trend in mean stride-length was 69 mm/year better following blind-active treatment than after placebo (Fig. 4). The size of effect was similar before and after excluding those receiving background antiparkinsonian medication. The effect was largely attributable to improvement following eradication (Table 3, $p = .005$ and $.007$, respectively).

At deblinding, there was no significant treatment effect on time-trends in ratio of torque to extend to flex the forearm or in ratings of stance-walk videos (Table 3). An isolated and unexplained increase in resistance to flexion (44% per year, $p = .007$) after blind-active was lost in the overall analysis of eradication success.

Body sway decreased by 9% per year following placebo compared with active, a time-trend perhaps echoed in a 16% (NS) decrease with failure of eradication compared with success (Table 3). This is in line with the decrease in sway in early parkinsonism [15]. Otherwise, there was no treatment effect on postural measures.

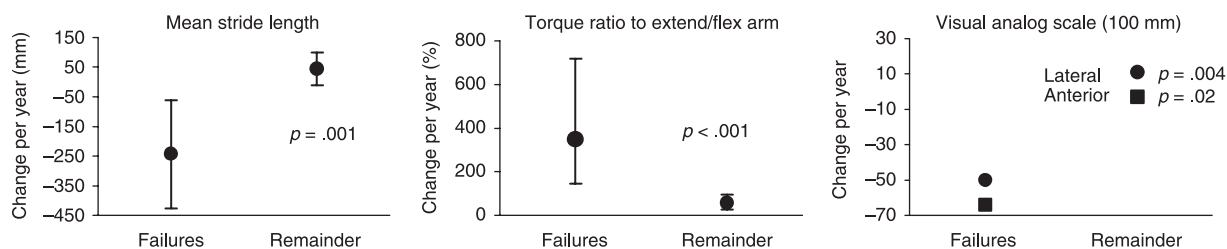


Figure 3 Objective and visual-analog documentation of deterioration with failure of *H. pylori* eradication relative to success. Estimated change over a year in stride-length, ratio of torque to extend to flex forearm, and ratings of stance-walk videos are contrasted between subjects in whom eradication failed ($n = 4$) and the remainder ($n = 16$). Mean (95% CI) is given for objective measures, point estimate for ratings.

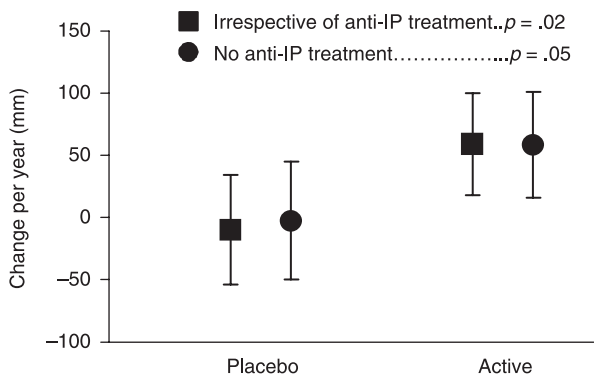


Figure 4 Time trends in primary outcome following double-blind active and placebo *H. pylori* eradication therapy. Estimated change (95% CI) in mean stride-length over a year is shown, including ($n = 18$) and excluding ($n = 13$) those on background antiparkinsonian (IP) medication. Two subjects in whom blind-active treatment failed are excluded. These and five others were receiving background medication.

Reaction time and cognitive efficiency were unaffected (Table 3). Weight was unaffected, no one being underweight at run-in.

Discussion

No disease has been attributed exclusively to *H. pylori*. Thus, if *H. pylori* can cause idiopathic parkinsonism, one should not demand its presence in every case [33]. In the Western populations, in general, any cohort that is followed seems to demonstrate a loss of *H. pylori*. This is thought to be due to the widespread and repeated use of antimicrobials, even low eradication rates having a cumulative effect. However, given our eradication failure rate ($\geq 4/20$) with “counsel of perfection” therapy, accidental iatrogenic eradication is perhaps less likely in those destined to be labeled parkinsonian. The disparity between immunoblot and EV or UBT positivity in parkinsonism could represent memory of past infection, persistent organism in low density or as a “dormant” form, or persistent antigen. In human immunodeficiency virus (HIV) infection, reduced prevalence of EV or UBT positivity, when the circulating CD4⁺ count is $< 100/\mu\text{l}$, was attributed to gastric mucosal atrophy [34,35]. Lymphopenia was not so marked here, atrophy a minor feature.

Isolating *H. pylori* from probands, and culturing it, is axiomatic to the efficacy study. Meeting Koch’s third postulate, by reproducing parkinsonism through inoculation into a healthy host, is a stumbling-block. Idiopathic parkin-

sonism is described only in man, and has a long latency. We have employed an alternative classical approach to defining an environmental insult. Spouses cohabiting with probands for half a century showed differences from control couples in objectively measured brady/hypokinesia, rigidity, postural abnormality, and psychomotor performance [14,16]. Knee-jerk attribution of these, plus the shift in lymphocyte count, to learned or reactive behavior, or selective mating, seems naïve. Although most *H. pylori* infections are acquired in childhood, spousal transmission is documented [36]. Even so, time lag in acquisition, plus differing propensities for “burning-out” or “damping-down,” would militate against finding concordance between partners in *Helicobacter* status.

Koch was not in a position to perform an antimicrobial intervention experiment. Failure of *H. pylori* eradication therapy in idiopathic parkinsonism was associated with alarming worsening of the syndrome. A 29 cm/year decrease in mean stride-length, with failure relative to success, is disabling, as is a threefold increase in the ratio of resistance to extend the forearm over that to flex. When increased axially and in limbs, the ratio describes the characteristic flexed posture. An archive of videos allowed independent overview. Even the 7 cm/year relative increase in stride-length after blind-active intervention is encouraging, particularly since the effect was independent of background antiparkinsonian therapy. Idiopathic parkinsonism is naturally progressive, gait precisely regulated. Overall, affecting the time-trend in three cardinal signs (brady/hypokinesia, rigidity and postural abnormality) implies an effect on parkinsonism, not simply on well-being. The natural experiment of failed *H. pylori* eradication suggests that any benefit accompanying successful eradication was not through elimination of a different microbe.

Idiopathic parkinsonism has diverse presentations and natural history. Removing a driving infection may not ameliorate all facets. The most favorable model would be that production of toxins, which have caused cumulative damage over decades, ceases. These might be inflammatory mediators produced by the host [37] or toxic/pharmacologically active molecules from the organism. A mechanism, such as restoration of neurotrophic support by glia following their deactivation, may not be organism-specific, the importance of *Helicobacter* being its chronicity. Whether eradication of, say, chronic periodontal disease would also ameliorate parkinsonism is a

pertinent question, but more difficult to address. A less favorable model would be that *Helicobacter* triggers autoimmunity. Killing it may be too little too late, but phenotype and natural history could be modified [5]. Marked deterioration, where eradication failed, might be due to release of a bolus of (possibly auto-reactive) antigen from killed bacteria, aggravating the effect of ongoing infection.

Gastric inflammatory response to *H. pylori* is important in determining the 10–15% of infected individuals who go on to peptic ulcer or gastric malignancy [38]. Regulation of systemic inflammation and immunity, by host genes and bacterial virulence factors, might prove equally important in crossing the diagnostic threshold for idiopathic parkinsonism. *H. pylori* culture supernatants and whole bacteria inhibit human T cell proliferation [39], as do *H. pylori*-specific regulatory T-lymphocytes [40]. However, the downward shift in lymphocyte count in parkinsonism was irrespective of UBT evidence of current infection, and, indeed, more pronounced in the immunoblot-negative. It could be that blot-negativity itself results from the immune incompetence. Then, blot-positive status would grossly underestimate the numbers that are infected (albeit at “low density”), or have been infected. Once cytotoxic T cells and B cells are reduced in idiopathic parkinsonism [41], response to eradication therapy appears jeopardized [42].

In the longer term, those eradication successes receiving background antiparkinsonian medication improved the most. This may simply reflect a greater burden of reversible damage (i.e. more scope for improvement). Loss of antagonism (e.g. by inflammatory products) to, or improved absorption of, antiparkinsonian medication, may contribute. Improved absorption should have an early effect on outcome, but increase in stride-length was no greater in those receiving antiparkinsonian medication in the double-blind phase. Moreover, in a case of late parkinsonism [37], a striking U-turn in performance resulted from successful *Helicobacter* eradication and gross drug-induced dyskinesia became minimal. Improved absorption would have increased dyskinesia rather than creating this favorable dichotomy.

Autoimmunity has been discussed as a potential mechanism for idiopathic parkinsonism [43], and, with particular reference to *cagA*-positivity, for extra-alimentary manifestations of *H. pylori* infection [44]. Idiopathic thrombocytopenic purpura might arise from mimicry between CagA

and platelet antigens, and arteriosclerotic plaque instability from cross-reactivity with CagA and VacA [44]. Persistence of serum CagA antibodies, in the absence of urease-B and VacA antibodies, appears not only predictive for idiopathic parkinsonism, but also a bad prognostic sign [11]. Moreover, all failures of eradication, with their rapidly progressive parkinsonism, were CagA seropositive. Outcome of successful eradication might prove better in CagA negative probands [4/16]. Indeed, eradication of *Helicobacter heilmannii* (which does not possess *cagA* gene) in a CagA seronegative subject, had marked long-term benefit on idiopathic parkinsonism [37]. Thus, although implicated, CagA does not appear a prerequisite for *Helicobacter*-associated parkinsonism.

Objective quantification of the facets of parkinsonism has proven invaluable in tracking disease progression, homing in on insults, and documenting the interplay of disease and antiparkinsonian medication [12,14,15]. The present tight efficacy study has provided pathogenetic and therapeutic clues, beyond the scope of intention-to-treat trials. Clearly, the interim analysis needs to be consolidated in the longer term in all 30 probands, and effects reproduced, taking into account geographical differences in host and microbial factors. Whether suppression of *H. pylori* by treatment of other infections (e.g. urinary tract) would have the same effect on the time course as failed anti-*Helicobacter* therapy is of practical importance. Our limited experience with anti-*Helicobacter* therapy in idiopathic parkinsonism where there is gastritis, but no evidence of the organism [37], certainly does not justify “blunderbuss” antimicrobial therapy, as trialed in Alzheimer’s disease [45]. However, a targeted approach may be relevant in the overlap conditions of Alzheimer’s disease and depression. The low serum vitamin B₁₂ in these conditions [46,47] could result from *Helicobacter*-associated autoimmune atrophic gastritis. Although some atrophy was seen in a third of efficacy study subjects, parkinsonism has not been distinguished from other neurological diseases by cerebrospinal fluid B₁₂ concentration [48].

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References

- 1 Calne DB, Snow BJ, Lee C. Criteria for diagnosing Parkinson's disease. *Ann Neurol* 1992;32(suppl): S125-7.
- 2 Calne DB. Parkinson's disease is not one disease. *Parkinsonism Relat Disord* 2001;7:3-7.
- 3 Strang RR. The association of gastro-duodenal ulceration with Parkinson's disease. *Med J Aust* 1965;52:842-3.
- 4 Atschuler E. Gastric *Helicobacter pylori* infection as a cause of idiopathic Parkinson's disease and non-arteric anterior optic ischaemic neuropathy. *Med Hypotheses* 1996;47:413-4.
- 5 Dobbs SM, Dobbs RJ, Weller C, Charlett A. Link between *Helicobacter pylori* infection and idiopathic parkinsonism. *Med Hypotheses* 2000;55:93-8.
- 6 Charlett A, Purkiss AG, Dobbs SM, Weller C, Peterson DW. Association of circulating TNF- α and IL-6 with ageing and parkinsonism. *Acta Neurol Scand* 1999;100:34-41.
- 7 Drumm B, Perez Perez GI, Blaser MJ, Sherman PM. Intrafamilial clustering of *Helicobacter pylori* infection. *N Engl J Med* 1990;322:358-63.
- 8 de la Fuente-Fernandez R, Calne DB. Evidence for environmental causation of Parkinson's disease. *Parkinsonism Relat Disord* 2002;8:235-41.
- 9 Dobbs RJ, Charlett A, Dobbs SM, Weller C, Peterson DW. Parkinsonism: Differential age-trend in *Helicobacter pylori* antibody. *Alim Pharm Ther* 2000;14:1199-205.
- 10 Charlett A, Dobbs RJ, Dobbs SM, Weller C, Brady P, Peterson DW. Parkinsonism: Siblings share *Helicobacter pylori* seropositivity and facets of syndrome. *Acta Neurol Scand* 1999;99:26-35.
- 11 Weller C, Charlett A, Oxlade N, et al. Role of chronic infection and inflammation in the gastrointestinal tract in the etiology and pathogenesis of idiopathic Parkinsonism Part 3: predicted probability and gradients of severity of idiopathic parkinsonism based on *H. pylori* antibody profile. *Helicobacter* 2005;10:288-97.
- 12 Bowes SG, Dobbs RJ, Henley M, et al. Objective evidence for tolerance, against a background of improvement, during maintenance therapy with controlled release levodopa/carbidopa. *Eur J Clin Pharmacol* 1992;43:483-9.
- 13 Weller C, O'Neill CJA, Charlett A, et al. Defining small differences in efficacy between anti-parkinsonian agents using gait analysis: A comparison of two controlled release formulations of levodopa/decarboxylase inhibitor. *Br J Clin Pharmacol* 1993;35:379-85.
- 14 Kirolos C, O'Neill CJA, Dobbs RJ, et al. Quantification of the cardinal signs of parkinsonism and of associated disability in spouses of sufferers. *Age Ageing* 1993;22:20-6.
- 15 Kirolos C, Charlett A, Bowes SG, et al. Time course of physical and psychological responses to selegiline monotherapy in newly diagnosed, idiopathic parkinsonism. *Eur J Clin Pharmacol* 1996;50:7-18.
- 16 Kirolos C, Charlett A, O'Neill CJA, et al. Objective measurement of activation of rigidity: Diagnostic, pathogenetic and therapeutic implications in parkinsonism. *Br J Clin Pharmacol* 1996;41:557-64.
- 17 Weller C, Humphrey SJE, Kirolos C, et al. Gait on a shoestring: Falls and foot separation in Parkinsonism. *Age Ageing* 1992;21:242-4.
- 18 Charlett A, Weller C, Dobbs SM, Dobbs RJ. Breadth of base whilst walking: Effect of ageing and parkinsonism. *Age Ageing* 1998;27:49-54.
- 19 Dobbs RJ, Bowes SG, Charlett A, et al. Hypothesis: The bradyphrenia of parkinsonism is a nosological entity. *Acta Neurol Scand* 1993;87:255-61.
- 20 Folstein MF, Folstein SE, McHugh PR. Minimal state. A practical method for grading the cognitive state of patients for the clinician. *J Psychiat Res* 1995;12:189-98.
- 21 Bowes SG, Clark PK, Leeman AL, et al. Determinants of gait in the elderly parkinsonian on maintenance levodopa/carbidopa therapy. *Br J Clin Pharmacol* 1990;30:13-24.
- 22 Lin LI-K. A concordance correlation coefficient to evaluate reproducibility. *Biometrics* 1989;45:255-68.
- 23 Glupczynski Y, Mégraud F, Lopez-Brea M, Andersen LP. European multicentre survey of in

- vitro antimicrobial resistance in *Helicobacter pylori*. *Eur J Clin Microbiol Infect Dis* 2001;20:820–3.
- 24 Andrews J, ed. BSAC Disc Diffusion Method for Antimicrobial Susceptibility Testing Report 2.1.4. Birmingham: British Society for Antimicrobial Chemotherapy, 2003.
 - 25 McNulty C, Owen R, Tompkins D, et al. *Helicobacter pylori* susceptibility testing by disc diffusion. *J Antimicrobial Chemotherapy* 2002;49:601–9.
 - 26 Elviss NC, Owen RJ, Xerry J, Walker AM, Davies K. *Helicobacter pylori* resistance patterns and genotypes in adult dyspeptic patients from a regional population in North Wales. *J Antimicrobial Ther* 2004;54:435–40.
 - 27 Chisholm SA, Owen RJ. Development and application of a novel screening PCR assay for direct detection of '*Helicobacter heilmannii*'-like organisms in human gastric biopsies in Southeast England. *Diagnostic Microbiol Infect Dis* 2003;46:1–7.
 - 28 Dixon MF, Genta RM, Yardley JH, Correa P, and the Participants in the International Workshop on the Histopathology of gastritis, Houston 1994. Classification and grading of gastritis: the updated Sydney System. *Am J Surg Path* 1996;20:1161–81.
 - 29 Diggle PJ, Heagerty P, Liang K-Y, Zeger SL. Random effects models. In: Analysis of Longitudinal Data, 2nd edn. Oxford Statistical Science Series, 25. Oxford: Oxford University Press, 2000; 169–89.
 - 30 Rabe-Hesketh S, Pickles A, Anders S. GLLAMM Manual. Technical Report 2001/01. London: Department of Biostatistics and Computing Institute of Psychiatry, King's College, University of London, 2001.
 - 31 Stata Base Reference Manual, (vol. 3). N-R, Release 8. College Station, Texas: Stata Press, Stata Corporation, 2003.
 - 32 Cohen J. A coefficient of agreement for nominal scales. *Ed Psychol Measurements* 1960;20:37–46.
 - 33 Wang TC, Fox JG. *Helicobacter pylori* and gastric cancer: Koch's postulates fulfilled? *Gastroenterology* 1998;115:642–8.
 - 34 Lichter M, Lorenz C, Nischalke HD, Scheurlen C, Sauerbtuch T, Rockstroh JK. Decreased prevalence of *Helicobacter pylori* infection in HIV patients with AIDS defining diseases. *Z Gastroenterol* 2002;40:11–4.
 - 35 Fabris P, Pilotto A, Bozzola L, et al. Serum pepsinogen and gastrin levels in HIV-positive patients: Relationship with CD4⁺ cell count and *Helicobacter pylori* infection. *Aliment Pharmacol Ther* 2002;16:807–11.
 - 36 Parente F, Maconi G, Sangaletti O, et al. Prevalence of *Helicobacter pylori* infection and related gastro-duodenal lesions in spouses of *Helicobacter pylori* positive patients with duodenal ulcer. *Gut* 1996;35:629–33.
 - 37 Dobbs RJ, Dobbs SM, Weller C, et al. Role of chronic infection and inflammation in the gastrointestinal tract in the etiology and pathogenesis of idiopathic parkinsonism part 1: eradication of *Helicobacter* in the cachexia of idiopathic parkinsonism. *Helicobacter* 2005;10:267–75.
 - 38 Del Giudice G, Michetti P. Inflammation, immunity and vaccines for *Helicobacter pylori*. *Helicobacter* 2004;9:23–8.
 - 39 Schmees C, Sander M, Volland P, et al. *H. pylori* activates T-cells while inhibiting their proliferation. *Helicobacter* 2004;9 (Suppl. 1):522.
 - 40 Lundgren A, Suri-Payer E, Enarsson K, Svennerholm A-M, Lundin BS. *Helicobacter pylori*-specific CD4⁺ CD25^{high} regulatory T cells suppress memory T-cell responses to *Helicobacter pylori* in infected individuals. *Infect Immun* 2003;71:1755–62.
 - 41 Bas J, Calopa M, Mestre M, et al. Lymphocyte populations in Parkinson's disease and in rat models of parkinsonism. *J Neuroimmunol* 2001;113:146–52.
 - 42 Borody T, Ren Z, Pang G, Clancy R. Impaired host immunity contributes to *Helicobacter pylori* eradication failure. *Am J Gastroenterol* 2002;97:3032–7.
 - 43 Hunot S, Hirsh EC. Neuroinflammatory process in Parkinson's disease. *Ann Neurol* 2003;53 (Suppl.):S49–60.
 - 44 Gasbarrini A, Carloni E, Gasbarrini G, Chisholm SA. *Helicobacter pylori* and extragastric diseases – Other helicobacters. *Helicobacter* 2004;9:57–66.
 - 45 Loeb MB, Molloy DW, Smieja M, et al. A randomized controlled trial of doxycycline and rifampicin for patients with Alzheimer disease. *J Am Geriatr Soc* 2004;52:381–7.
 - 46 Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B₁₂, and serum total homocysteine levels in confirmed Alzheimer disease. *Arch Neurol* 1998;55:1449–55.
 - 47 Pennix BW, Guralnik JM, Ferrucci L, Freid LP, Allen RH, Stabler SP. Vitamin B₁₂ deficiency and depression in physically disabled older women. Epidemiologic evidence from the Women's Health and Ageing Study. *Am J Psychiatry* 2000;157:715–21.
 - 48 Baig SM, Qureshi GA, Minami M. The interrelation between the deficiency of vitamin B₁₂ and neurotoxicity of homocysteine with nitrite in some neurologic disorders. *Biogenic Amines* 1998;14:1–14.