

ORIGINAL ARTICLE

The impact of atopic disease on the risk of post-infectious fatigue and irritable bowel syndrome 3 years after *Giardia* infection. A historic cohort study

GUNNHILD S. HUNSKAR¹, NINA LANGELAND², KNUT-ARNE WENSAAS^{1,3}, KURT HANEVIK^{2,4}, GEIR EGIL EIDE^{5,1}, KRISTINE MØRCH^{4,6} & GURI RORTVEIT^{1,3}

¹Department of Public Health and Primary Health Care, University of Bergen, Norway, ²Institute of Medicine, University of Bergen, Norway, ³Research Unit for General Practice, Uni Health, Uni Research, Bergen, Norway, ⁴National Centre for Tropical Infectious Diseases, Haukeland University Hospital, Bergen, Norway, ⁵Centre for Clinical Research, Haukeland University Hospital, Bergen, Norway, and ⁶Unit for Infectious Diseases, Department of Medicine, Haukeland University Hospital, Bergen, Norway

Abstract

Objective. To investigate whether atopic disease influences the prevalence of irritable bowel syndrome (IBS) and chronic fatigue (CF) after giardiasis. **Methods.** A questionnaire was sent to all confirmed cases of giardiasis after a Norwegian outbreak, with response rate of 65.3% (817/1252). Controls were randomly selected matched on age and sex, with response rate of 31.4% (1128/3598). Associations were evaluated by use of logistic regression analyses. **Results.** In the *Giardia* exposed group, 47.8% of those with asthma had IBS compared with 45.3% in those without asthma ($p = 0.662$). For controls, corresponding percentages were 23.9% and 12.2% ($p < 0.001$). Among those with asthma, the adjusted relative risk (RR) for IBS was 2.03 (95% confidence interval (CI): 1.45, 2.62) for the exposed group compared with controls. In those without asthma, the corresponding RR was 3.80 (95% CI: 3.30, 4.32). In the exposed group, 51.5% of those with asthma had CF compared with 44.9% in those without asthma ($p = 0.218$). For controls, corresponding percentages were 19.3% and 10.7% ($p = 0.004$). Among those with asthma, the adjusted RR for CF was 2.73 (95% CI: 1.98, 3.45) for the exposed compared with controls. In those without asthma, the corresponding RR for CF was 4.25 (95% CI: 3.66, 4.85). **Conclusion.** For the exposed, having asthma or allergy did not increase the outcome of IBS or CF. For the control group, having an atopic disease made a substantial risk difference, with significantly more IBS and CF.

Key Words: allergy, asthma, chronic fatigue, *Giardia lamblia*, irritable bowel syndrome

Introduction

An epidemic of giardiasis occurred in Bergen, Norway in 2004, caused by contamination of the city center's main water reservoir. Approximately 48,000 persons were exposed to the contaminated water and an estimated 2500 people received metronidazol [1]. An outbreak of this size is rare in Europe, and presumably, most of the affected individuals had not been previously exposed to the parasite as the prevalence of *Giardia lamblia* is low in the Nordic countries [2].

The main symptoms of acute giardiasis are diarrhea, nausea, abdominal pain, malabsorption and weight loss. It is usually not presented as a severe condition, and first-line treatment against *Giardia* is easily available.

The research group has previously demonstrated a strong association between giardiasis and both chronic fatigue (CF) and irritable bowel syndrome (IBS) 3 years after the infection [3]. Previous studies have demonstrated an association between asthma and allergy with IBS or chronic fatigue syndrome

Correspondence: Gunnhild S. Hunskar, Department of Public Health and Primary Health Care, Kalfarveien 31, N-5018 Bergen, Norway. Fax: +47 55586130. E-mail: gunnhild.hunskar@student.uib.no

(Received 30 March 2012; revised 22 April 2012; accepted 21 May 2012)

ISSN 0036-5521 print/ISSN 1502-7708 online © 2012 Informa Healthcare
DOI: 10.3109/00365521.2012.696681

(CFS) [4,5], and after the outbreak in Bergen in 2004 there were reports from clinicians that asthma and allergy seemed to be linked to persisting abdominal symptoms.

The aim of this study was to investigate if asthma or allergy affected the association between giardiasis and the outcomes of IBS and CF.

Methods

Study design and participants

This was a historic cohort study 3 years after the outbreak of giardiasis in 2004. Questionnaires were sent by mail to all 1252 patients who had a positive fecal test for *Giardia* during the outbreak, and a control group of 3598 individuals matched by age and sex. The control group was randomly selected among inhabitants in Bergen, and they were included unless they had had giardiasis during the outbreak. The details on establishing the study groups have been published previously [3].

Variables

The exposure in this study is the detection of *Giardia* in stool samples during the autumn of 2004.

The main outcome variables are IBS and CF 3 years after the *Giardia* infection. For the IBS variable, the authors used the ROME III diagnostic criteria [6], recording abdominal pain or discomfort of certain regularity linked to alterations in bowel movements. The questions needed to diagnose IBS were translated into Norwegian by the research group [3]. To measure fatigue, the authors employed the fatigue questionnaire developed by Chalder et al. [3,7]. The authors had previously published results with regards to the primary outcomes of IBS and CF, showing that the prevalence of IBS was significantly higher in *Giardia* exposed individuals (46.1%) than in controls (14.0%) ($p < 0.001$). The prevalence of CF was also significantly higher among the *Giardia* exposed (46.1%) than in controls (12.0%) ($p < 0.001$).

The questions about asthma have been used in previous epidemiological studies, but were somewhat modified for this study [8]. Asthma was defined as confirmatory answer to the question: "Have you or have you had asthma?" Follow-up questions were: "If yes, has this been confirmed by a medical doctor?" and "Have you used any asthma medication in the last month (sprays, inhalations of different kinds or tablets)?" The asthma group in the material was defined by including everyone answering "yes" to the entry question ($n = 219$). Those who, despite answering "no" to this question, had answered that

asthma had been diagnosed by a medical doctor were also included ($n = 1$). Those who answered "uncertain" to the entry question were included if the diagnosis had been confirmed by a medical doctor or they had used asthma medication in the last month ($n = 14$). In total, 234 were defined as having asthma. Allergy was defined by affirmative answer to one question: "Have you or have you had hay fever or nasal allergy?", with answering options, "yes", "uncertain" and "no".

Statistical analyses

The χ^2 test was applied to test differences between proportions. Confounders evaluated were age, sex, work, income and level of education. Confounding and interactions were evaluated by logistic regression modeling [9]. Odds ratios (OR) were the original outcome measure given in the analyses. These were transformed into relative risks (RR) by use of the formula developed by Zhang and Yu [10]. As matching can sometimes harm efficiency in non-experimental cohort studies although it introduces no bias, all analyses were done adjusted for the matching. As the 1:2 matching did not work out perfectly, this was carried out with crude conditioning on gender and age rather than on the specific matched patient-control sets to utilize all responders. Moreover, work, income and education were also adjusted for. In line with the primary aims of this report, modifying effects of asthma and allergy on the association between *giardiasis* and the presence of IBS or CF were examined by including and testing corresponding interaction terms in the logistic regression models. Strength and significance of associations are given by the RR and its 95% confidence interval (CI). The likelihood ratio test for interaction is significant if the RRs (i.e. ORs) between the outcomes and *Giardia* infection differ between the atopy groups.

Ethical approval

This study has been approved by the Regional Committee for Medical and Health Research Ethics (project 150.07), and by the Ombudsman for Privacy in Research, Norwegian Social Science Data Services (project 17014).

Results

The overall response rate was 40.1% (1945/4850), with a 65.3% (817/1252) response rate among exposed and 31.4% (1128/3598) among controls as presented previously [3]. The mean age in the study population was 36.2 years with 65.7% being females.

In those receiving the questionnaire among the exposed, the mean age was 34.7 and 61.1% were females. In controls receiving the questionnaire, the mean age was 34.0 and 59.4% were females. The patient and control groups were similar in age and sex distribution, as expected from the design of the study (Table I). The proportion of individuals not married or with higher education was significantly higher among exposed than controls [3].

The overall prevalence in the study population was 12.0% for asthma, 26.5% for allergy and 6.1% for combined asthma and allergy. Table I shows that the prevalence of allergy was significantly higher in the exposed group than in the control group. The prevalence of asthma was similar in the two groups.

Table II shows the prevalence of IBS among exposed and controls depending on atopic status. The prevalence of IBS was similar among the *Giardia* exposed with asthma (47.8%) and without asthma (45.3%) ($p = 0.662$). The prevalence of IBS was significantly higher among controls with asthma (23.9%) as compared with controls without asthma (12.2%) ($p < 0.001$). In adjusted analyses for participants with asthma, the RR for IBS was 2.03 (95% CI: 1.45, 2.62) for those who had been exposed to *Giardia* as compared with the control group. In those without asthma, the RR for IBS was 3.80 (95% CI: 3.30, 4.32) for those who had been exposed to *Giardia* as compared with the control group. For the outcome of IBS, the likelihood ratio test for interaction was not statistically significant ($p = 0.075$).

The prevalence of IBS was similar among *Giardia* exposed individuals with allergy (45.9%) and without allergy (43.7%) ($p = 0.595$). The prevalence of IBS was 17.3% in controls with allergy and 12.1% in controls without allergy ($p = 0.031$). In the adjusted analyses for subjects with allergy, the RR for IBS was 2.68 (95% CI: 2.10, 3.27) for those exposed to *Giardia* as compared with the control group. For those without allergy the RR for IBS was 3.73 (95% CI: 3.13, 4.34) for those exposed to *Giardia* as compared with the control group. For the outcome of IBS, the likelihood ratio test for interaction was not statistically significant ($p = 0.330$).

Table III shows the prevalence of CF among patients and controls depending on atopic status. The prevalence of CF among *Giardia* exposed was 51.5% for those with asthma and 44.9% for those without asthma ($p = 0.218$). The prevalence of CF in controls was significantly different with almost the double prevalence in those with asthma (19.3%) compared with those without asthma (10.7%) ($p = 0.004$). In adjusted analyses for participants with asthma, the RR for CF was 2.73 (95% CI: 1.98, 3.45) for those who had been exposed to *Giardia* as compared with the control

group. In those without asthma, the RR for CF was 4.25 (95% CI: 3.66, 4.85) for those who had been exposed to *Giardia* compared with the control group. For the outcome of CF, the likelihood ratio test for interaction was not statistically significant ($p = 0.462$).

The prevalence of CF among *Giardia* exposed individuals with allergy was 49.8% as compared with 42.9% in those without allergy ($p = 0.089$). The prevalence of CF was 13.7% in controls with allergy and 11.0% in the controls without allergy ($p = 0.232$). In the adjusted analyses for subjects with allergy, the RR for CF was 3.64 (95% CI: 2.87, 4.42) for those exposed to *Giardia* as compared with the control group, and the corresponding RR was 4.00 (95% CI: 3.33, 4.68) for those without allergy. For the outcome of CF, the likelihood ratio test for interaction was not statistically significant ($p = 0.993$).

Discussion

The authors have previously shown a strong association between acute giardiasis and IBS or CF 3 years later [3]. In the current study, the authors found that in the exposed group who had had giardiasis and with high prevalence of IBS, having asthma or allergy did not affect the prevalence of IBS significantly. In the control group, there was a statistically significant

Table I. Characteristics and demographics for 817 *Giardia* exposed and 1128 controls in Bergen, Norway 2004.

	<i>Giardia</i> exposed		Controls		p -Value ^a
	<i>n</i>	(%)	<i>n</i>	(%)	
Age groups					n.s.
0–19	39	(4.8)	36	(3.2)	
20–39	526	(64.4)	736	(65.2)	
40–59	187	(22.9)	276	(24.5)	
60–79	56	(6.9)	76	(6.7)	
80–99	9	(1.1)	4	(0.4)	
Females	540	(66.1)	738	(65.4)	n.s.
IBS	355	(46.1)	155	(14.0)	< 0.001
CF	366	(46.1)	134	(12.0)	< 0.001
Asthma	99	(12.2)	135	(12.1)	n.s.
Allergy	231	(28.8)	285	(26.1)	< 0.001
Asthma and allergy	52	(6.4)	66	(5.9)	n.s.
Marital status					0.004
Single	271	(33.5)	293	(26.1)	
Married	497	(61.4)	778	(69.3)	
Divorced/separated	33	(4.1)	41	(3.7)	
Widow/widower	9	(1.1)	11	(1.0)	
Education					0.004
Primary school	37	(4.7)	59	(5.3)	
Secondary school	169	(21.3)	308	(27.7)	
University	587	(74.0)	746	(67.0)	

Abbreviations: CF = chronic fatigue; IBS = irritable bowel syndrome; n.s. = not significant, $p > 0.05$.

^a p -Value from the χ^2 test.

Table II. Logistic regression analyses of IBS among an exposed group 3 years after giardiasis and a control group. Results are given stratified for asthma and allergy status (yes, no, uncertain).

Baseline characteristics		IBS	Unadjusted		Adjusted ^c	
		n (%)	RR	95% CI	RR	95% CI
Giardia vs. asthma interaction ^a						
Asthma	Control	32 (23.9)	1		1	
	<i>Giardia</i> exposed	43 (47.8)	2.00	(1.42, 2.59)	2.03	(1.45, 2.62)
No asthma	Control	114 (12.2)	1		1	
	<i>Giardia</i> exposed	291 (45.3)	3.72	(3.22, 4.22)	3.80	(3.30, 4.32)
Uncertain asthma	Control	8 (25.8)	1		1	
	<i>Giardia</i> exposed	19 (59.4)	2.30	(1.29, 3.14)	2.37	(1.35, 3.19)
Giardia vs. allergy interaction ^b						
Allergy	Control	49 (17.3)	1		1	
	<i>Giardia</i> exposed	100 (45.9)	2.65	(2.08, 3.24)	2.68	(2.10, 3.27)
No allergy	Control	86 (12.1)	1		1	
	<i>Giardia</i> exposed	190 (43.7)	3.61	(3.02, 4.21)	3.73	(3.13, 4.34)
Uncertain allergy	Control	18 (21.7)	1		1	
	<i>Giardia</i> exposed	57 (54.8)	2.53	(1.79, 3.22)	2.56	(1.82, 3.26)

Abbreviations: CI = confidence interval; OR = Odds ratio; RR = relative risk; IBS = Irritable bowel syndrome.

Likelihood ratio test is based on a test of OR. ORs have been transformed into RRs.

^aLikelihood ratio test for interaction, *p*-Value: 0.075.

^bLikelihood ratio test for interaction, *p*-Value: 0.330.

^cAdjusted for sex, age, asthma or allergy, and interactions between exposure status and asthma or allergy.

increase in prevalence of IBS for those with asthma or allergy. No large difference was seen according to asthma or allergy status among those with CF in the exposed group. In the control group, a relatively high difference in CF prevalence was found between those with asthma or allergy compared with those without asthma or allergy.

The relationship between giardiasis and long-term complications has been documented from this outbreak [3]. It is now known that there is an association between atopic disease and IBS or CF in the control group that is not present in the exposed group.

This study is based on a large outbreak, which gives more statistical power to comparisons than most previous studies on waterborne gastrointestinal infections. The exposed group included those giving a positive fecal test for *Giardia* during the outbreak of 2004. The risk of misclassification for individuals in this group is minimal. The control group was originally composed of two controls per exposed individual, matched for age and sex. This group was later extended because of low response. This resulted in a large control group (1128 individuals). The rarity of giardiasis in Norway makes it unlikely that controls had been exposed to giardiasis to such an extent that it would make an impact on the material in this study.

There are some limitations due to low response rates, especially in the control group, and some selection bias cannot be ruled out. In the exposed group, the response rate (65.3%) is high compared with other studies using mailed questionnaires [11]. There might still be selection bias as some of the exposed individuals may not feel the questions concerned them if they did not experience IBS or CF, while others might not answer the questionnaire due to bad health. Hence, bias may go both ways for the prevalence measure. In the control group, the response rate was 31.4% which is quite low and awareness of selection bias is necessary, especially for prevalence estimates. However, risk estimates for the impact of atopic disease are more robust to such bias. Another limitation is that it is not known when the participants got their atopic disease, before or after the *Giardia* infection, IBS or CF. Accordingly, inferences about causality cannot be made. This being an epidemiological study, the mechanisms behind the findings are not investigated.

In the general population in Norway, a study on fatigue showed a prevalence of 11–14% [12]. The present study showed a prevalence of 12% in the control group, indicating that the low response rate may not necessarily have created a problem of selection bias.

Table III. Logistic regression analyses of CF among an exposed group 3 years after giardiasis and a control group. Results are given stratified for asthma and allergy status (yes, no, uncertain).

Baseline characteristics		CF	Unadjusted		Adjusted ^c	
		n (%)	RR	95% CI	RR	95% CI
Giardia vs. asthma interaction ^a						
Asthma	Control	26 (19.3)	1		1	
	<i>Giardia</i> exposed	51 (51.5)	2.67	(1.93, 3.40)	2.73	(1.98, 3.45)
No asthma	Control	102 (10.7)	1		1	
	<i>Giardia</i> exposed	295 (44.9)	4.19	(3.61, 4.78)	4.25	(3.66, 4.85)
Uncertain asthma	Control	5 (16.7)	1		1	
	<i>Giardia</i> exposed	20 (58.8)	3.53	(1.83, 4.93)	3.58	(1.87, 4.97)
Giardia vs. allergy interaction ^b						
Allergy	Control	39 (13.7)	1		1	
	<i>Giardia</i> exposed	113 (49.8)	3.64	(2.87, 4.40)	3.64	(2.87, 4.42)
No allergy	Control	79 (11.0)	1		1	
	<i>Giardia</i> exposed	193 (42.9)	3.90	(3.25, 4.58)	4.00	(3.33, 4.68)
Uncertain allergy	Control	14 (16.1)	1		1	
	<i>Giardia</i> exposed	59 (55.7)	3.46	(2.40, 4.44)	3.47	(2.40, 4.45)

Abbreviations: CF = chronic fatigue; CI = confidence interval; RR = relative risk; OR = Odds ratio.

Likelihood ratio test is based on a test of OR. ORs have been transformed into RRs.

^aLikelihood ratio test *p*-Value: 0.462.

^bLikelihood ratio test *p*-Value: 0.993.

^cAdjusted for sex, age, asthma or allergy, and interactions between exposure status and asthma or allergy.

The authors used the Rome III diagnostic criteria to detect IBS. This is developed to investigate IBS and has been used previously for research purposes, but has not yet been formally validated [6]. To measure fatigue, the authors used a validated questionnaire, developed by Chalder et al. [7,13]. The study investigates CF, not CFS, which is a clinical diagnosis. Some of the patients with CF in this study most probably also have CFS, however, it is not known how many. The questions to investigate asthma or allergy have not been validated, but the questions for asthma have been used in previous studies [8]. In detection of asthma, three questions were asked. For allergy only one question was asked, and the uncertainty for this diagnosis is higher. The group that answered "uncertain" showed a pattern of results that was difficult to interpret.

A possible relationship between atopic disorders and IBS has been investigated previously. An increased prevalence of IBS among asthma patients as compared with controls has been shown [5,14]. In these studies, asthma patients and healthy controls were asked about IBS symptoms through questionnaires. The first study found an IBS prevalence of 41.3% in asthma patients (*n* = 150) compared with 20.8% in healthy controls (*n* = 120). Tobin et al.

presented the expression "atopic IBS" [15]. They used the Rome II criteria for detection of IBS in patients from three different clinics (gastroenterology clinic, allergy/immunology clinic and general medicine clinic). In the study, they showed that there was a higher prevalence of different atopic disorders among individuals with IBS than those without IBS. These studies conclude that there is an association between atopy and IBS. This is in line with the findings in the control group in the present study. None of the previous studies investigated the possible influence of an infectious disease. In the authors' exposed group, however, atopy did not seem to play a role. A possible interpretation is that the *Giardia* infection has such a large effect on the outcomes that the additional effect of atopy on IBS may become obliterated.

Associations between infections and IBS [11,16], and between infections and CFS [4,17] have previously been shown.

The relationship between atopic disorders and CF has been little investigated, except for a small uncontrolled study [4]. The present study has a large control group, and the results in this group may serve as a study of the relationship in a normal population. This part of the study suggests that there is a clear association between atopic disease and CF. However,

conclusions should be made with some caution due to the low response rate in the control group.

In this study, the authors have investigated post-infectious IBS and CF, and the association with asthma and allergy. The conclusion is that the prevalence of IBS and CF after giardiasis is not significantly influenced by the presence of atopic disease. One explanation might be that acute infections, like giardiasis, have the ability to trigger these complications in a high proportion of susceptible individuals, reaching a ceiling where no further effect of other co-factors such as atopy can be seen. The authors found striking associations on the other hand between atopy and IBS and CF in the control group. Such an association has been suggested previously, but should be more thoroughly investigated in the future.

Acknowledgements

The authors would like to thank Trygve Hausken, Per Olav Vandvik, Jon Håvard Loge and Cecilie Svanes for valuable input regarding the selection of study variables and design of the questionnaire. Funding: An open grant from the Municipality of Bergen covered the cost for production and mailing of the questionnaire and for the services delivered by Statistics Norway. All researchers are independent from the sponsors. The sponsors had no role in study design, in collection, analysis and interpretation of data, or in writing or deciding to submit the manuscript.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

- [1] Nygard K, Schimmer B, Sobstad O, Walde A, Tveit I, Langeland N, et al. A large community outbreak of water-borne giardiasis-delayed detection in a non-endemic urban area. *BMC Public Health* 2006;6:141.
- [2] Horman A, Korpela H, Sutinen J, Wedel H, Hanninen ML. Meta-analysis in assessment of the prevalence and annual incidence of *Giardia* spp. and *Cryptosporidium* spp. infections in humans in the Nordic countries. *Int J Parasitol* 2004;34:1337–46.
- [3] Wensaas KA, Langeland N, Hanevik K, Morch K, Eide GE, Rortveit G. Irritable bowel syndrome and chronic fatigue 3 years after acute giardiasis: historic cohort study. *Gut* 2012; 61:214–19.
- [4] Straus SE, Dale JK, Wright R, Metcalfe DD. Allergy and the chronic fatigue syndrome. *J Allergy Clin Immunol* 1988;81: 791–5.
- [5] Roussos A, Koursarakos P, Patsopoulos D, Gerogianni I, Philippou N. Increased prevalence of irritable bowel syndrome in patients with bronchial asthma. *Respir Med* 2003; 97:75–9.
- [6] Rome III Diagnostic Questionnaire for the Adult Functional GI Disorders. Available from <http://www.romecriteria.org/pdfs/AdultFuncGIQ.pdf>.
- [7] Chalder T, Berelowitz G, Pawlikowska T, Watts L, Wessely S, Wright D, et al. Development of a fatigue scale. *J Psychosom Res* 1993;37:147–53.
- [8] Grassi M, Villani S, Marinoni A. Classification methods for the identification of 'case' in epidemiological diagnosis of asthma. *Eur J Epidemiol* 2001;17:19–29.
- [9] Kleinbaum DG, Klein M. Logistic regression: a self-learning text. 2nd ed. New York: Springer-Verlag; 2002.
- [10] Zhang J, Yu KF. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. *JAMA* 1998;280:1690–1.
- [11] Mearin F, Perez-Oliveras M, Perello A, Vinyet J, Ibanez A, Coderch J, et al. Dyspepsia and irritable bowel syndrome after a *Salmonella* gastroenteritis outbreak: one-year follow-up cohort study. *Gastroenterology* 2005;129:98–104.
- [12] Loge JH, Ekeberg O, Kaasa S. Fatigue in the general Norwegian population: normative data and associations. *J Psychosom Res* 1998;45:53–65.
- [13] Morriss RK, Wearden AJ, Mullis R. Exploring the validity of the Chalder Fatigue scale in chronic fatigue syndrome. *J Psychosom Res* 1998;45:411–17.
- [14] Huerta C, Garcia Rodriguez LA, Wallander MA, Johansson S. Risk of irritable bowel syndrome among asthma patients. *Pharmacoepidemiol Drug Saf* 2002;11:31–5.
- [15] Tobin MC, Moparty B, Farhadi A, DeMeo MT, Bansal PJ, Keshavarzian A. Atopic irritable bowel syndrome: a novel subgroup of irritable bowel syndrome with allergic manifestations. *Ann Allergy Asthma Immunol* 2008;100: 49–53.
- [16] Thabane M, Marshall JK. Post-infectious irritable bowel syndrome. *World J Gastroenterol* 2009;15:3591–6.
- [17] Hickie I, Davenport T, Wakefield D, Vollmer-Conna U, Cameron B, Vernon SD, et al. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. *BMJ* 2006;333:575.