SHORT REPORT

Comparative study of preceding *Campylobacter jejuni* infection in Guillain-Barré syndrome in Japan and The Netherlands

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Abstract

A comparative study was made in Japan and The Netherlands of the presence of preceding *Campylobacter jejuni* infections in Guillain-Barré syndrome (GBS). It was conducted in two laboratories using different serological criteria. The Japanese results showed no significant difference in the frequency of *C jejuni* infection between the Japanese (17/88, 19%) and Dutch (21/132, 16%) patients with GBS. The Dutch investigation showed a higher frequency in Dutch patients (45/132; 34%) than in Japanese patients (20/88; 23%), but the difference did not reach significance. Although the frequencies of preceding *C jejuni* infection have been reported to be higher in Asian countries than in western countries, the findings of this collaborative study show that the incidence of antecedent *C jejuni* infection in GBS in Japan is not higher than in The Netherlands and that serological assays vary considerably between laboratories.

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Guillain-Barré syndrome (GBS) is an immune mediated neuropathy, which occurs world wide, affecting patients of all ages and both sexes. Two thirds of patients with GBS have histories of antecedent infectious illness, and the gram negative bacterium *Campylobacter jejuni* has emerged as the most common antecedent infectious agent in GBS.¹ Serological studies showed that the frequency of prior *C jejuni* infection in GBS ranges from 17% to 66%,² the frequencies in northern China (66%)² and Japan (45%)³ being higher than those in western countries such as the United States (17%, 36%),^{4,5} the United Kingdom (26%),⁶ The Netherlands (32%),⁷ Germany (39%),⁸ and Australia (38%).⁹ The reports suggest that in Asian countries GBS is more closely associated with *C jejuni* than it is in western countries. These studies, however, used different assay systems, making it difficult to compare the incidence of preceding *C jejuni* infection in GBS. A cooperative project by

Dokkyo University and Erasmus University was planned to compare the frequencies of *C jejuni* associated GBS in Japan and The Netherlands. Serological examinations of the incidence of antecedent *C jejuni* infection in the same serum samples from Japanese and Dutch patients with GBS were made in two laboratories, after which the frequency of *C jejuni* infection and the correlation between the two different serological assays were analyzed.

Patients and methods

PATIENTS

Pretreatment serum samples were obtained from patients with GBS in Japan and The Netherlands, all of whom met the established criteria for GBS.¹⁰ The samples were stored at -80°C until used. The Japanese GBS group consisted of 88 consecutive patients who had been referred to the Neuroimmunological Laboratory at Dokkyo University in 1998, from university hospitals and district general hospitals throughout Japan. None of them had been included in our previous study.¹¹ The Dutch patients consisted of 132 of 147 patients with GBS who had participated in the Dutch GBS trial,¹² 15 patients being excluded because no serum was available. These patients did not differ from the others in clinical manifestations and the course of the disease. Control serum samples were available from patients with other neurological diseases (27 Japanese and 42 Dutch) and from healthy volunteers (56 Japanese and 30 Dutch). There were no significant differences in the ages and sex of the patients with GBS, other neurological diseases, or of the healthy controls in Japan and The Netherlands.

C JEJUNI SEROLOGY

Antecedent *C jejuni* infection was serologically examined in the two laboratories by enzyme linked immunosorbent assays (ELISAs) as reported elsewhere.^{11,13} These ELISAs are routinely used in each country for testing prior *C jejuni* infection in GBS. The antigen protein for ELISA used at Dokkyo University (Japanese ELISA)¹¹ was prepared from a *C jejuni* O:19 strain isolated from a Japanese patient with GBS and used at 100 ng protein/well. Serum was considered positive when anti-*C jejuni* IgG

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Figure 1 Frequency (%) of serological evidence of recent *C jejuni* infection in two laboratories: (A) Japan and (B) The Netherlands. Solid bar, Japanese population; open bar, Dutch population; ELISA=enzyme linked immunosorbent assay; GBS=Guillain-Barré syndrome; OND=other neurological diseases; HC=healthy controls.

titres were 2000 or more in the Japanese ELISA. The cut off was defined based on the findings for 17 patients with GBS, from whom *C jejuni* had been isolated, and findings for 46 healthy controls. This system has sensitivity of 82% and specificity of 88%, and the presence of anti-*C jejuni* IgG antibody alone provided sufficient evidence of recent *C jejuni* infection.¹¹ Serum samples from the Japanese subjects were first used for the Japanese ELISA then immediately frozen at -80°C and with ample dry ice air lifted to The Netherlands for testing by The Netherlands ELISA.¹³ Serum samples from Dutch subjects were treated similarly. In The Netherlands ELISA, the antigen protein was prepared from a *C jejuni* Lau 48 strain isolated from a patient with enteritis and used at 300 ng protein/well. Serological evidence of recent *C jejuni* infection was defined as the presence of anti-*C jejuni* IgM, or IgA antibodies, or both.¹³ The Japanese and Dutch investigators were blinded to the diagnoses for each other's patients and serological results during the testing.

STATISTICAL ANALYSIS

The Spearman rank correlation test was used to test the association between the Japanese and Netherlands' ELISAs. The difference in the frequency of positive *C jejuni* serology between these ELISAs was tested by the Mantel-Haenszel procedure. Other differences between groups were examined by the χ^2 or Fisher's exact test where appropriate. Differences in medians were examined by the Mann-Whitney *U* test. A difference was considered significant at $p < 0.05$. All statistical analyses were made with Statcel® software (OMS, Saitama, Japan).

Results

JAPANESE ELISA

Serological evidence of recent *C jejuni* infection was found in 17 (19%) of the 88 Japanese patients with GBS and 21 (16%) of the 132 Dutch patients with GBS (fig 1). There was no significant difference between the Japanese and

Dutch patients with GBS, those with other neurological diseases, or healthy controls. In both the Japanese and Dutch populations, the frequency of positive *C jejuni* serology was higher in the patients with GBS than in the patients with other neurological diseases or in the healthy controls, but this difference was significant only for Japanese patients with GBS and the healthy controls ($p = 0.0001$).

NETHERLANDS ELISA

The incidence of positive *C jejuni* serology in the Dutch patients with GBS (45/132; 34%) was higher than in the Japanese patients with GBS (20/88; 23%) ($p = 0.07$) (fig 1). No significant differences were found between the Japanese (15%) and Dutch (5%) patients with other neurological diseases or between the Japanese (18%) and Dutch (10%) healthy controls. Dutch patients with GBS had positive *C jejuni* serology significantly more often than the healthy controls ($p = 0.009$) and those who had other neurological diseases ($p = 0.0002$), whereas in the Japanese population, there was only a slight, non-significant difference in *C jejuni* serology among the patients with GBS (23%), those with other neurological diseases (15%), and the healthy controls (18%).

COMPARISON OF ELISA SYSTEMS

C jejuni serology results showed good correlations in the Japanese ($p < 0.0001$, $r = 0.77$) and Dutch ($p < 0.0001$, $r = 0.76$) populations. Opposite results for both ELISAs were seen in 64 (17%) of 375 serum samples included in this study (table 1). Most of these samples were positive in The Netherlands ELISA but

Table 1 Comparison of *C jejuni* serology results from the two laboratories

	Japanese ELISA	
	Positive	Negative
The Netherlands ELISA:		
Positive	31 (8%)	53 (14%)
Negative	11 (3%)	280 (74%)

ELISA=Enzyme linked immunosorbent assay.

negative in the Japanese assay. The frequency of positive results was higher in The Netherlands (22%) than in the Japanese ELISA (11%) ($p=0.00003$).

Discussion

Isolation of *C jejuni* from the faeces of a patient is the most specific method for determining *C jejuni* enteritis. Because GBS develops only 1–3 weeks after diarrhoea and the excretion period of the bacteria is limited, by this criterion a large proportion of patients with GBS related to *C jejuni* would be negative. The incidence of antecedent gastrointestinal symptoms does not always reflect the frequency of preceding *C jejuni* infection in GBS because asymptomatic *C jejuni* infections are frequent and probably differ by country.¹⁴ By contrast, serodiagnostic methods are suitable for investigating preceding *C jejuni* infection between countries.

We performed serological assays on the same serum samples in two widely separated laboratories. Both ELISA systems showed that the incidence of *C jejuni* serology does not differ in Japanese and Dutch patients with GBS, but the true incidence of preceding *C jejuni* infection in GBS is still not clear. Based on Japanese ELISA results, we reported elsewhere a 31% frequency of antecedent *C jejuni* infection in Japanese patients with GBS,¹¹ higher than that found in the present study. Every assay was standardised using well characterised serum with high anti-*C jejuni* antibody activity, and this discrepancy therefore may be due to the difference in patient populations surveyed in the previous and present studies. Because the population in the present study consisted of consecutive patients, we think that the Japanese patients in the present study are representative of the total group of Japanese patients with GBS.

Results of the assay systems routinely used in the two countries' laboratories were well correlated. The Netherlands ELISA, however, showed a higher frequency of positive results. Because all 11 Japanese patients with GBS from whom *C jejuni* had been isolated were seropositive in The Netherlands ELISA (not included in this study), this discrepancy seems to reflect the different definition of positive serology, not the difference of the antigen preparation. In the Dutch patients with GBS, in particular, there was a great difference in seropositive frequency (34% in The Netherlands' ELISA, 16% in the Japanese ELISA). This indicates that the incidence of preceding *C jejuni* infection in GBS varies markedly with the assay's sensitivity and that it is difficult to compare the incidences of *C jejuni* related GBS that were obtained by serological methods in previous studies. It is noteworthy that a high

frequency of positive serology was found for Japanese patients with other neurological diseases (15%) and the healthy controls (18%) by The Netherlands ELISA, indicative that specificity of an assay used to determine *C jejuni* serology may depend not only on the antigen preparation used but on the origin of the test population.

In conclusion, our comparative study showed that there is no significant difference in the incidence of preceding *C jejuni* infection in patients with GBS in Japan and in The Netherlands. Differences between laboratories indicate that any worldwide comparative investigation of *C jejuni* serology should be done in one laboratory and that the assay methods should be improved for yielding higher sensitivity and more specificity irrespective of the laboratory.

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