Diagnosis of *Bacteroides fragilis* infection with counter-immunoelectrophoresis

O. A. OKUBADEJO, N. F. LIGHTFOOT¹, AND W. G. HEWITT

From the Public Health Laboratory, St. Mary's General Hospital, Portsmouth, Hants, UK

SUMMARY In a study of 188 patients and 109 controls, the detection of antibody by counter-immunoelectrophoresis was used as a diagnostic aid in human infections with *Bacteroides fragilis*. It was found that positive results indicated current infection and negative results were not conclusive. The method used was simple, rapid, and easily performed in a routine laboratory, but further work is needed to enhance antigen potency.

Bacteroides fragilis strains are often cultured from routine bacteriological specimens in laboratories with adequate anaerobic technique (Leigh, 1974; Peach, 1975). At present the organisms are the most frequent cause of non-sporing Gram-negative anaerobic infection in man. During the past five years the bacteria have been isolated with increasing frequency in many laboratories. Nevertheless the pathogenicity of the isolates is often questioned because the bacteria are a major component of normal body flora (Quick, 1972; Topley, 1975; Willis, 1975). A foul-smelling discharge usually indicates anaerobic infection, but this sign may be absent in certain lesions in which *B. fragilis* is a likely infective agent. These include superficial wounds, bed sores, diabetic ulcers, salpingitis, pelvic inflammatory disease, vaginitis, episiotomy wounds, and otitis. In these infections the pathogenic role of *B. fragilis* can be ascertained either by a good response to treatment with metronidazole or by the finding of specific antibodies in the patient's serum. Recent reports indicate that antibodies can be demonstrated in sera by agglutination, haemagglutination, immunodiffusion, and immunofluorescent methods (Danielsson *et al.*, 1972; Quick *et al.*, 1972; Rissing *et al.*, 1974). The reports show that IgG immunoglobulins are probably more important than IgM in the diagnosis of bacteroides infection. IgG antibodies can be detected efficiently and quickly by counter-immunoelectrophoresis (World Health Organisation, 1973); so far as we know, this technique has not been applied to the detection of antibodies in *B. fragilis*. We report a study using this method.

Patients and methods

INFECTED PATIENTS' TEST SERA

Sera were taken from patients suspected of having bacteroides infection because of positive cultures or persistent, foul-smelling, purulent lesions suggesting anaerobic infection. Initial specimens of serum were usually obtained within three days of the isolation of *B. fragilis*, and subsequent serum samples were taken whenever possible at an interval of about 7-10 days.

CONTROL SERA

Sera were also collected from patients who had no evidence of infection but had had *B. fragilis* in high vaginal swabs before a gynaecological operation (uninfected patients). Other control sera were obtained from healthy young male naval recruits and healthy female blood donors.

All sera used in this study were taken from blood given for other purposes.

COUNTER-IMMUNOELECTROPHORESIS (CIEP)

Tests were performed using Shandon Southern U77 electrophoretic apparatus with a Vokam DC power supply. The electrophoresis gel consisted of 0.75% agarose and 0.75% Noble agar (Difco) in barbitone buffer containing 40 g barbitone and 26 g sodium acetate dissolved in 4 litres of distilled water and adjusted to pH 8.6. This buffer was also used in the electrophoretic tank; 6 ml of molten electrophoretic agar was layered onto clean 50 × 75 mm glass slides and allowed to set. Wells, 4 mm in diameter and 7 mm apart, were cut in the gel. Antigen and serum were applied to the cathodal and anodal wells respectively. A constant current of 20 mA per slide was passed through the agar gel for 90 minutes. The current was then switched off, and the slide was

¹Surgeon Lieutenant Commander, Royal Navy

Received for publication 29 March 1978

examined for precipitin lines using incident light against a dark background.

ANTIGEN

The antigen used was prepared from the two strains of *B. fragilis* found in preliminary tests to have the widest range of antigen component (Okubadejo and Allen, unpublished work). The two strains were stored in cooked meat medium at room temperature and subcultured on Diagnostic Sensitivity Test (DST Oxoid) agar plates enriched with 10% lysed horse blood, and incubated anaerobically for 48 hours. After purity checks, the organisms were harvested from the surface of the plates and washed four times in normal saline by centrifugation at 3400 *g* for 25 minutes in a refrigerated centrifuge at a temperature of not more than 10°C. The washed organisms were suspended in barbitone buffer at pH 8.6, the final concentration being equivalent to approximately 20% wet weight per volume. The bacteria were then disrupted by an ultrasonic disintegrator (MSE) operating with an amplitude of 6.8 microns peak to peak at 22 kHz for a total time of 15 minutes; cooling was provided by an ice-bath. Disruption of the cells was monitored by Gram stain.

SPECIMENS, CULTURE, AND IDENTIFICATION OF BACTERIA

Bacteria were isolated from routine clinical specimens including pus, blood cultures, wound swabs, high vaginal swabs, and body fluids. The specimens were cultured on blood agar and MacConkey agar and incubated aerobically; on DST Oxoid agar containing 10% lysed horse blood and 10 mg per litre gentamicin (DST-gentamicin); and on a blood agar plate without antimicrobials for anaerobic incubation. Cooked meat medium freshly prepared in this laboratory was also inoculated and incubated. After 48 hours' incubation at 37°C the plates were examined, and the cooked meat was subcultured onto blood agar and DST-gentamicin agar, which were incubated anaerobically for 48 hours. Anaerobiosis was obtained in Baird-Tatlock jars, the air having been replaced with a mixture of 95% hydrogen and 5% carbon dioxide. Aerobic bacteria were identified by methods described by Cowan and Steel (1974), and anaerobic bacteria as in the *Anaerobic Laboratory Manual* (Holdeman and Moore, 1972). Gas liquid chromatographic analysis of the products of glucose metabolism was also used for identification.

Patients from whom *Bacteroides* species other than *B. fragilis* were isolated were not included in this study.

Results

INFECTED PATIENTS (Table 1)

One hundred and eighty-eight patients were considered to have clinical evidence of bacteroides infection. *B. fragilis* was cultured from the lesions of 149 patients; cultures from the remaining 39 were negative.

Table 1 Comparison of CIEP results in infected patients and controls

Infected patients	CIEP		
	Positive (%)	Negative (%)	Total
Cultures for <i>B. fragilis</i>			
Positive	98 (65.8)	51 (34.2)	149
Negative	7 (17.9)	32 (82.1)	39
Total	105 (55.9)	83 (44.1)	188
Controls			
Uninfected patients	0 (0)	22 (100)	22
Healthy women	1 (2)	49 (98)	50
Healthy young men	1 (2.7)	36 (97.3)	37
Total	2 (1.8)	107 (98.2)	109

CONTROLS

There were 109 controls consisting of 50 healthy female blood donors, 37 healthy young male naval recruits, and 22 preoperative gynaecological patients, from whom *B. fragilis* had been isolated but who had no clinical evidence of infection.

POSITIVE CIEP TESTS (Table 1)

One hundred and five (56%) of the 188 infected patients' sera gave positive CIEP tests compared with two (1.8%) of 109 controls. Of the 105 infected patients with positive CIEP, seven had negative *B. fragilis* cultures. Three of the seven had tubo-ovarian abscesses, and one had a lung abscess from which anaerobes were detected by gas chromatography of pus. The fifth patient had salpingitis and hydro-salpinx; the CIEP test was also positive on fluid collected from the pouch of Douglas during a pelvic laparoscopy. The other two had vaginal discharge with *Candida albicans* infection.

NEGATIVE CIEP TESTS (Table 1)

One hundred and seven (98.2%) of 109 controls had negative CIEP tests compared with 83 (44%) of 188 infected patients. However, 51 (61%) of these 83 patients had *B. fragilis* in their lesions, and 20 of the 51 patients also had definite clinical evidence of *B. fragilis* infection. Seven of the 20 patients had *B. fragilis* septicaemia, four had severely infected hysterectomy wounds, four had infections associated with cholecystectomies, three had extensive appendix wound abscesses, and one had a pyometra with an

intrauterine device *in-situ*.

The remaining 32 of the 83 patients with negative CIEP tests did not have *B. fragilis* in their lesions, and their infections could have been due to other organisms.

Table 2 gives details of the variety of clinical conditions from which the CIEP positive and negative sera were found, together with the results of cultures for *B. fragilis*.

TIMING OF DEMONSTRABLE ANTIBODY

As the majority of sera used in this study were taken from blood collected for other reasons, we were not able to determine the precise time relationship of antibody formation to clinical infection in many patients; however, we were able to relate them in

nine patients, and details are given in Table 3. Blood was obtained from four of the nine patients before the onset of infection, and none of these sera had demonstrable antibody. However, antibody was found in all nine sera seven to 10 days after the onset of infection, and the CIEP test remained positive for three to six weeks. All nine patients had chemotherapy, which could have modified the production of antibody.

The antibody detected with the CIEP test cannot be quantitated, and the conventional four-fold rise in titre was not demonstrable.

Discussion

Small amounts of natural antibodies, mainly IgM,

Table 2 Details of CIEP results: infected patients

Clinical condition	Bacteroides found in lesion		Bacteroides not found		Total number investigated
	CIEP		CIEP		
	+ ve	- ve	+ ve	- ve	
Appendicectomy abscess	4	2	0	0	6
Appendicectomy wound infection	4	1	0	0	5
Intestinal resection, colectomy, infected suture	2	0	0	0	2
Abdominoperineal resection, infected wound	4	2	0	4	10
Ischiorectal abscess, drainage	6	1	0	0	7
Pilonidal sinus, excision	0	1	0	0	1
Cholecystectomy:					
Pyrexia	1	2	0	0	3
Infected wound	4	1	0	1	6
Fracture left tibia, infected wound	1	0	0	0	1
Cellulitis leg, varicose ulcer	1	0	0	0	1
Infected leg ulcer, diabetic	2	0	0	0	2
Bed-sore sacral region	1	3	0	3	7
Otitis media, persistent ear discharge	2	1	0	0	3
Mediastinal abscess, thoracotomy and drainage	1	0	0	0	1
Lung abscess, empyema drainage, sputum	1	0	1	0	2
Nephrectomy, wound infection	2	0	0	0	2
Infected					
Sparkes-Mandril site	1	0	0	0	1
Infected hydrocoele, long history scrotal swelling	1	0	0	0	1
Septicaemia with positive blood cultures after:					
Colectomy	1	3	0	0	4
Cholecystectomy	0	1	0	0	1
Nephrectomy	1	0	0	0	1
Renal transplantation	0	3	0	0	3
Vaginal discharge	17	15	2	5	39
Infections in abdominal hysterectomy wounds:					
Pyrexia	7	1	1	1	10
Vaginal discharge	2	0	0	0	2
Intravenous site	4	0	0	2	6
Vaginal hysterectomy wounds	1	0	0	0	1
Postpartum pyrexia	4	2	0	2	8
Infected episiotomy	5	5	0	6	16
Postabortal infection:	10	3	0	5	18
Pyrexia	1	2	0	1	4
Discharge	1	0	0	1	2
Uterine infection, pyometra with intrauterine device	3	1	0	0	4
Salpingitis, hydrosalpinx	0	0	1	0	1
Wound infection after hysterectomy	1	1	0	0	2
Tubo-ovarian abscess, salpingo-oophorectomy with drainage of abscess	2	0	2	1	5
Total	98	51	7	32	188
		149		39	188

Table 3 CIEP results of sera taken before, during, and after an infection: 9 patients only

Clinical features	Serum			
	Before infection	1st after infection	2nd after infection	3rd after infection
Infected appendix wound. <i>B. fragilis</i> isolated 8th postop day	CIEP negative	10 days postop. CIEP positive	Not taken	Not taken
Appendix wound abscess. <i>B. fragilis</i> isolated. Treated with metronidazole	Not taken	10 days postop. CIEP positive	20 days postop. CIEP negative	Not taken
Cholecystectomy wound abscess. <i>B. fragilis</i> , <i>E. coli</i> , and <i>Proteus</i> sp found in abscess five days postop. Treated with gentamicin and clindamycin	CIEP negative	7 days postop. CIEP positive	Not taken	6 weeks postop. CIEP negative
Cholecystectomy. Postoperative septicaemia 5th day. Treated with metronidazole	CIEP negative	8 days postop. CIEP positive	16 days postop. CIEP positive	3 weeks postop. CIEP positive
Total abdominal hysterectomy Infected wound and pyrexia <i>B. fragilis</i> and <i>E. coli</i> in wound 9th day. Treated with cotrimoxazole and gentamicin	CIEP negative	12 days postop. CIEP positive	19 days postop. CIEP positive	6 weeks postop. CIEP negative
Tubo-ovarian abscess. <i>E. coli</i> and <i>B. fragilis</i> in abscess swab at laparotomy. Treated with gentamicin and clindamycin	Not taken	3 days postop. CIEP positive	10 days postop. CIEP positive	4 weeks postop. CIEP negative
Persistent vaginal discharge. IUD <i>in situ</i> . Pure growth <i>B. fragilis</i> . Treated with metronidazole	Not taken	3 days after isolation <i>B. fragilis</i> CIEP positive	2 weeks after isolation <i>B. fragilis</i> CIEP negative	Not taken
Persistent vaginal discharge. <i>B. fragilis</i> isolated. Treated with clindamycin	Not taken	3 days after isolation <i>B. fragilis</i> CIEP positive	10 days after isolation <i>B. fragilis</i> CIEP positive	Not taken
Persistent vaginal discharge. Pure growth <i>B. fragilis</i> . Treated with metronidazole	Not taken	3 days after isolation <i>B. fragilis</i> CIEP positive	4 weeks after isolation <i>B. fragilis</i> CIEP negative	Not taken

are found in normal human serum using highly sensitive methods such as haemagglutination and immunofluorescent tests (Danielsson *et al.*, 1972; Quick *et al.*, 1972; Rissing *et al.*, 1974). The antibody detected by the CIEP test is mainly IgG (Lightfoot, 1976), and our findings indicate that it can detect specific *B. fragilis* antibodies in the serum of patients with active infection. Antibody was detected in 105 (56%) of 188 patients with clinical evidence of infection, and *B. fragilis* was found in all but seven of the 105 patients. The control group included 22 patients from whom *B. fragilis* had been isolated but who showed no clinical evidence of infection; antibody was not detected in any of them. These findings indicate that when specific antibody was found the patients had evidence of infection, but the mere isolation of *B. fragilis* from specimens was not directly related to infection.

There were 83 patients with clinical evidence of infection, 51 of whom had positive cultures, in whom no antibody was detected. There are three possible explanations for this. Firstly, the serum may have been taken at too early a stage of the infection, and this may be one reason for the negative CIEP result in seven of our nine patients with *B. fragilis* septicaemia. It was possible to determine the time relationship of antibody formation to infection in only nine of our patients, but the evidence suggested that antibodies could be detected seven to 10 days

after the onset of infection. Secondly, antibody formation may have been modified in some patients by either chemotherapy or immunosuppressive therapy. Lastly, it is possible that some antigenic components are missing from the standard *B. fragilis* strains from which we prepared our antigen; the production of a more efficient antigen might result in a higher detection rate. The antigen used in our tests was a whole-cell antigen, which was likely to be bacteroides genus-specific, as described by other authors (Shinjo *et al.*, 1971; Sonnenwirth, 1973; Hofstad, 1977).

In conclusion, therefore, we suggest that the CIEP test, which can be performed easily in most routine laboratories and is quick and inexpensive, can be of use in confirming the diagnosis of *B. fragilis* infection. Using the technique described here, a positive result confirmed infection in over half the cases, including some from whom the organism was not isolated; a negative result was inconclusive. With further improvements in the antigen, and a more precise knowledge of the time relationship of infection to antibody production, the technique may eventually prove useful in an even higher proportion of cases.

References

Cowan, S. T., and Steel, K. J. (1974). *Manual for the*

- Identification of Medical Bacteria*, 2nd edition. Cambridge University Press, London.
- Danielsson, D., Lambe, D. W. Jr., and Persson. S. (1972). The immune response in a patient to an infection with *Bacteroides fragilis* ss. *fragilis* and *Clostridium difficile*. *Acta Pathologica et Microbiologica Scandinavica Sect B*, **80**, 709-712.
- Hofstad, T. (1974). Antibodies reacting with lipopolysaccharides from *Bacteroides melaninogenicus*, *Bacteroides fragilis*, and *Fusobacterium nucleatum* in serum from normal human subjects. *Journal of Infectious Diseases*, **129**, 349-352.
- Hofstad, T. (1977). Cross-reactivity of *Bacteroides fragilis* O antigens. *Acta Pathologica et Microbiologica Scandinavica*, **B**, **85B**, 9-13.
- Holdeman, L. V., and Moore, W. E. C. (Eds). (1972). *Anaerobic Laboratory Manual*. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.
- Leigh, D. A. (1974). Clinical importance of infections due to *Bacteroides fragilis* and role of antibiotic therapy. *British Medical Journal*, **3**, 225-228.
- Lightfoot, N. (1976). *M.Sc. Thesis, London University*.
- Peach, Susan (1975). Antibiotic-disc tests for rapid identification of non-sporing anaerobes. *Journal of Clinical Pathology*, **28**, 388-391.
- Quick, J. D. (1972). *Ph.D. Thesis, University of Missouri*.
- O. A. Okubadejo, N. F. Lightfoot, and W. G. Hewitt
- Quick, J. D., Goldberg, H. S., and Sonnenwirth, A. C. (1972). Human antibody to *Bacteroidaceae*. *American Journal of Clinical Nutrition*, **25**, 1351-1356.
- Rissing, J. P., Crowder, J. G., Smith, J. W., and White, A. (1974). Detection of *Bacteroides fragilis* infection by precipitin antibody. *Journal of Infectious Diseases*, **130**, 70-73.
- Shinjo, T., Beerens, H., Wattre, P., and Romond, C. (1971). Classification sérologique de 131 souches de *Bacteroides* du groupe *fragilis* (*Eggerthella*). *Annales de l'Institut Pasteur de Lille*, **22**, 85-100.
- Sonnenwirth, A. C. (1973). Serology of *Bacteroidaceae*. Abstract 1. *International Congress on Bacteriology, Jerusalem*, **1**, 183.
- Topley, W. W. C. (1975). *Topley and Wilson's Principles of Bacteriology, Virology and Immunity*. 6th edition revised by G. S. Wilson and A. Miles. Volume 1, p. 646. Arnold, London.
- Willis, A. T. (1975). A view of *Bacteroides*. *Journal of Antimicrobial Chemotherapy*, **1**, 254-255.
- World Health Organisation (1973). Viral hepatitis: report of a WHO scientific group. *Technical Report Series*, **512**.
- Requests for reprints to: Dr O. A. Okubadejo, Public Health Laboratory, St. Mary's General Hospital, East Wing, Milton Road, Portsmouth, PO3 6AQ, UK