

ANTIBODIES AGAINST PLEUROPNEUMONIA-LIKE ORGANISMS IN PATIENTS WITH SALPINGITIS*

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Since the first report of Dienes and Edsall (1937), a number of communications have recorded the isolation of pleuropneumonia-like organisms (PPLO) from the genital tract of the human female, especially in association with inflammatory diseases such as Bartholinitis, pelvic abscess, and salpingitis (Melén and Odeblad, 1951; Randall, Stein, and Ayres, 1950; Dienes, Ropes, Smith, Madoff, and Bauer, 1948; Klieneberger-Nobel, 1945, 1959, 1962). In only a few cases has a search for specific antibodies been undertaken. Beveridge, Campbell, and Lind (1946) found antibody in a proportion of the human sera they examined, but were unable to correlate the occurrence of antibody with any clinical condition. Stokes (1955) detected antibody against PPLO in four patients (two with puerperal sepsis, one with pyosalpinx, and one with empyema); PPLO but no other pathogens were isolated from the blood, genital tract, or operation wound. Stokes (1959) reported a further case of infection following panhysterectomy, in which PPLO were isolated from the blood and antibody was present in high titre. Melén and Gotthardson (1955) found high titres of PPLO antibody in five patients with salpingo-oophoritis.

Card (1959) examined serologically 56 PPLO strains isolated from the human genital tract and found that they belonged to one broad serological group; she distinguished them serologically from PPLO isolated from the mouth, from sewage, and from various animals. This serological type-specificity of the genital strain made possible a survey of a large number of sera in terms of antigens from a single representative PPLO strain. Sera from patients with venereal infections, non-venereal diseases, healthy blood donors, and children were examined. Female patients from venereal disease clinics had

the highest incidence of antibodies (44.5 per cent.), and female blood donors and children had the lowest (3.7 and 0 per cent. respectively). Sera from a few patients with salpingitis were included in Card's survey. The presence of antibody in most of these sera suggested that a fuller investigation of similar sera might elucidate the association of PPLO with the disease by correlating some clinical features with the distribution of PPLO antibody. No attempt was made to obtain specimens for culture. Indeed it would have been possible only in the few that were operated upon and in these patients prior treatment with broad-spectrum antibiotics made cultural examination of little value. Moreover, cultural examination of cervical swabs even in untreated patients would provide little information about the significance of PPLO in salpingitis, since PPLO are frequently found in the genital tract of female patients attending V.D. clinics (Klieneberger-Nobel, 1959).

Material and Methods

Sera were tested by the complement-fixation method (CFT). The preparation and standardization of the PPLO antigens, the titration of complement, and the test itself were carried out as described by Card (1959). Human sera known to give positive and negative reactions with human genital type 1 PPLO were included as controls with each batch of sera tested. Titres are expressed as the reciprocal of the highest serum dilution at which reaction occurred, and sera failing to react at $\frac{1}{10}$ are regarded as negative.

It was not possible to use the representative human genital Strain 56 used by Card. Antigen preparations of this strain were found to react non-specifically to high titre with rabbit antisera prepared against rat and other animal PPLO previously found to be serologically distinct from the human genital strains. It is

* Received for publication June 4, 1962.

probable that Strain 56 had lost its more specific antigens during the storage at -25°C . or during the repeated subculture necessary for maintaining the strain since its use by Card.

In this laboratory some seventy strains of PPLO isolated from the human genital tract have now been found by complement-fixation to belong to one broad serological group, but they were not completely identical antigenically (Card, 1959). Moreover, Oates, Whittington, and Wilkinson (1959), in complement-fixation tests on human sera, noted that antigen made from a strain of PPLO isolated from a patient with Reiter's disease was more sensitive in detecting PPLO antibodies in human sera than that made from a strain isolated from non-gonococcal urethritis.

In view of these finer antigenic differences within the human genital group, for the present survey antigens were prepared from three different strains:

H34, isolated by Dr. E. J. Stokes (University College Hospital) from an infected abdominal wound following hysterectomy;

H27, isolated from the urethral discharge of a man with acute Reiter's disease;

MJW, a strain kindly sent by Dr. M. J. Whittington of the London Hospital, and used by her as an antigen in complement-fixation tests for detecting PPLO antibodies in human sera.

All three strains belonged to the single broad serological group. With H34 antiserum the homologous titre was 10,240, with H27 2,560, and with MJW it was 5,120. With a 56 antiserum prepared by Card, H34, H27, and MJW all reacted to a titre of 1,280 compared with the homologous titre of 2,560. Both "fresh" (F) and "boiled" (B) antigens were prepared from these strains for titration against the human serum specimens. About half the sera were also titrated against an F antigen from strain PG27, designated as a "human genital type 2" strain, and kindly sent by Dr. D. G. ff Edward.

The specimens were obtained from 51 patients seen at the V.D. clinics or in the wards of the Central Middlesex Hospital and St. Mary's Hospital, London; 23 were diagnosed as having gonococcal salpingitis and 28 as having "non-specific" (non-gonococcal) salpingitis. Other types of salpingitis were not represented. As a rule there was a clinical history of acute lower abdominal pain which commonly had its onset following a monthly period. The body temperature varied and was quite a good index of severity. On vaginal examination, pain and tenderness was present in the fornices and one or both tubes were felt to be enlarged. A characteristic feature was severe pain on gentle lateral movement of the cervix. About three-quarters of the patients had an abun-

dant vaginal discharge. In all patients bacteriological and serological tests for gonorrhoea were carried out and the vaginal secretions were examined for *Trichomonas vaginalis* and *Monilia*. Patients in whom gonococci were found were treated with 4 to 6 mega units of procaine penicillin intramuscularly, and if there was no adequate clinical response of the salpingitis within a week, they received Achromycin by mouth, 250 mg. four times a day for 6 days. A few patients were also given a similar course of chloramphenicol. Patients in whom gonococci were not found were treated with Achromycin or chloramphenicol only. Patients who showed little or no clinical response a month after the start of treatment were given a further course of Achromycin combined with local short-wave diathermy. Sera were taken from 38 patients at an early stage of the acute illness, and from thirteen patients sera were first obtained during convalescence (more than 4 weeks after onset) or after a symptom-free interval of 2 to 12 months; from seventeen of the 51 patients it was possible to take a number of specimens over a period of time.

Results

In 51 patients with salpingitis the sera of thirty (58.8 per cent.) contained antibody for at least one of the three strains of human genital type 1 PPLO. The percentage of positives given by the three strains MJW, H34, and H27, was 55, 51, and 49 per cent. respectively.

These three strains belonged to the same broad serological group as the single Strain 56 used by Card (1959). Thus, the incidence of positive reactions in our salpingitis patients can be compared with that in the healthy female blood donors examined by Card. Even with the least sensitive strain, H27, 49 per cent. of salpingitis patients were positive compared with under 4 per cent. of female blood donors (Table I).

TABLE I
SERUM ANTIBODIES AGAINST HUMAN GENITAL TYPE 1
PPLO IN SALPINGITIS PATIENTS AND FEMALE BLOOD
DONORS

Group	No. Tested	PPLO Strain used as Antigen	Positive CFT	
			No.	Per cent.
Female Blood Donors	109	56	4	3.70
Salpingitis Patients	51	MJW	28	54.90
		H34	26	50.98
		H27	25	49.01

Some sera reacted with both F and B antigens, whereas others reacted only with either F or B (Table

II). The sera from salpingitis patients, like the human sera examined by Card (1959), reacted with F antigen more frequently than with B. Nevertheless, the small number of "B only" reactions suggests that B as well as F antigens should be included in any survey of human sera using the complement-fixation method. In seven of the eleven serum specimens reacting with both F and B antigens, the F titre was 2- to 4-fold higher than the B titre, and in four it was the same.

TABLE II

REACTIONS OF POSITIVE SERA WITH F AND B ANTIGENS

Positive Reactions with:	No. of Patients	No. of Serum Specimens
F Only	15	23
B Only	6	12
F and B	9	11

Most of the positive sera reacted with all three of the F or B antigens (Table III). Ten of the eleven sera reacting with only one or two of the F antigens did so with MJW, whereas only four were positive with H27 and three with H34. This shows that MJW is the most sensitive F antigen for detecting PPLO antibody in human serum. Of the eight sera reacting with only one or two of the B antigens, five were positive with H34, four with MJW, and three with H27; thus there was little difference between the sensitivities of the B antigens.

TABLE III

NUMBER OF SERUM SPECIMENS REACTING WITH ANTIGENS OF STRAINS H34, H27, AND MJW

Positive Reaction with:	Type of Antigen	
	F	B
H34 and H27 and MJW	23	15
H34 and H27	0	2
H27 and MJW	4	1
H34 and MJW	2	1
H34 Only	1	2
H27 Only	0	0
MJW Only	4	2
Total Number of Positive Sera ..	34	23

Against the three F antigens, the titres of any one serum differed no more than 2-fold, except in two sera in which the titres differed 4-fold. The widest range of B titres was also 4-fold, and this was in one serum only.

Thus, the titres obtained with the three different PPLO strains generally confirmed one another. Moreover, the percentage of positives given with each of the three strains (Table I) shows that few positives would have been missed even if the least sensitive strain, H27, had been used. The results suggest that there are minor antigenic differences,

possibly quantitative, among strains belonging to the same human genital group.

For the analysis of the clinical results (Tables IV, V, VII, and VIII), only the 25 patients whose sera reacted with antigens of the least sensitive strain, H27, have been considered positive. Table III shows that sera reacting with H27 reacted also with MJW or H34 or with both.

There is no significant difference in the distribution of PPLO antibodies in gonococcal and non-gonococcal salpingitis (Table IV).

TABLE IV

COMPLEMENT-FIXATION TEST (CFT) AGAINST PPLO IN GONOCOCCAL AND NON-GONOCOCCAL SALPINGITIS

Type of Salpingitis	Total No. of Patients	Complement-fixation Test	
		Positive	Negative
Gonococcal	23	10	13
Non-Gonococcal	28	15	13

It is noteworthy that penicillin or tetracycline was effective in promptly clearing the gonococci in the 23 patients with gonococcal salpingitis, but the subsequent course of the salpingitis was similar to that of the non-gonococcal patients who also received antibiotic treatment. There was an apparent prompt response of the salpingitis to antibiotic treatment in about two-thirds of the cases in each group and the remaining third had persistent or recurrent signs and symptoms 3 months or more after onset. The incidence of positive serological reactions was not influenced by antibiotic treatment (Table V).

TABLE V

INCIDENCE OF POSITIVE CFT IN RELATION TO RESPONSE TO TREATMENT

Condition	Treatment Response to Broad-Spectrum Antibiotics	No. of Patients	No. with Positive CFT
Salpingitis with Gonococci	Good	16	7
	Poor or None ..	7	3
Salpingitis without Gonococci ..	Good	18	9
	Poor or None ..	10	6

Trichomonas vaginalis was found in fourteen patients, four of whom gave a positive CFT. Monilia were present in three patients with vaginitis, all of whom also had PPLO antibody. Klieneberger-Nobel (1959) isolated PPLO from two of six patients with trichomonal vaginitis, showing that *Trichomonas* infestation can be accompanied by PPLO infection.

From seventeen of the patients more than one serum specimen was taken. All the specimens from

six of these patients were negative, and those from nine others consistently positive. In the two remaining cases, the earlier specimens were negative, the later positive: in one patient the change occurred within 25 days, the second specimen reacting with five out of six antigens; in the other patient the second sample, taken 2 months after the first, was positive with only one antigen. These were the only two patients in which rising titres were demonstrated; in neither case was the rise as great as that found by Melén and Gotthardson (1955) in patients with salpingo-oophoritis.

Six specimens from the patient with the highest antibody response observed showed a steady decrease in the F titres from 640 to 20-40 over a period of 13 months. Melén and Gotthardson (1955) reported a similar drop from a high convalescent titre (256-512) in two patients over a period of 2 years; in our patient the B titres were 20 to 80 at first, and became negative within 3½ months. F titres do not necessarily persist longer than B titres; in another patient a low B titre persisted for 4 months. The titres of seven other patients with consistently positive sera did not change more than 2-fold over periods ranging from 17 days to 12 months. We have insufficient evidence to relate the persistence of the F or B titres to any aspect of the disease such as chronicity. The persistence of positive titres in patients re-examined within one month was noted by Card (1959). Seven of our patients had positive titres persisting for periods between 2 and 13 months. Thus a single observation of a low positive titre may be evidence of an infection which occurred some months previously. There is also the possibility that, since the salpingitis is recurrent in some patients, PPLO can produce a chronic low-grade infection with exacerbations at intervals and persistence of antibodies.

The antibody titres for the 51 salpingitis patients are set out in Table VI. Where the F and B titres or the titres with the three antigens of the same type differed, the highest titre obtained is recorded. In more than half the positive reactors the titres were 10 to 20, like the majority of titres recorded by Card (1959) in V.D. clinic patients.

TABLE VI

DISTRIBUTION OF SERUM TITRES AGAINST HUMAN GENITAL TYPE 1 PPLO IN PATIENTS WITH SALPINGITIS

Titre	<10 (negative)	10	20	40	640	Total
No. of Patients. .	21	11	11	7	1	51

There is a significantly higher proportion of positives among patients with severe salpingitis than

among those with a mild or chronic condition (Table VII).

TABLE VII

INCIDENCE OF POSITIVE CFT IN RELATION TO SEVERITY OF SALPINGITIS

Degree of Severity of Salpingitis	No. of Patients	Complement-fixation Test	
		Positive	Negative
Mild or Moderate ..	28	12	16
Severe	19	12	7
Convalescent	4	1	3
Total	51	25	26

The highest proportion of positives occurs among patients examined 2 to 3 weeks after onset (Table VIII). Stokes (1955) found that positive titres were not detectable in the first 10 days after the onset of symptoms and that the highest titres occurred 14 to 28 days after the onset.

TABLE VIII

INCIDENCE OF POSITIVE CFT IN RELATION TO DURATION OF SALPINGITIS

Duration of Salpingitis (wks)	No. of Patients	Complement-fixation Test	
		Positive	Negative
1	22	11	11
2	8	6	2
3	2	2	—
4	6	3	3
8	9	2	7
12+	4	1	3
Total	51	25	26

29 serum specimens from eighteen patients were also tested against the F antigen of PG27, but none gave a positive reaction. Strain PG27 and two other so-called "human genital type 2" strains, 07 and Campo, obtained from Dr. H. E. Morton (Philadelphia), are serologically indistinguishable from the rat polyarthritis strain Jasmin, originally obtained from rat tumour tissue (Lemcke, unpublished). The significance of these "type 2" strains in human disease is obscure. Their isolation from man has never been reported in the United Kingdom, but we have isolated a strain of PPLO, serologically identified as a mouse strain, from the external genitalia of a man. It is probable that "non-human" types such as this mouse strain and PG27 occur on human genitalia as saprophytic contaminants.

Discussion

The presence of antibody, as detected by complement-fixation with our most sensitive human genital PPLO strain, in 55 per cent. of patients with salpingitis compared with less than 4 per cent. in healthy

female blood donors, suggests that PPLO are associated with this disease. Gonococci were present in just under half of the patients and the proportion of positive complement-fixation tests against PPLO was similar in gonococcal and non-gonococcal salpingitis. This is in accord with the findings of Melén and Gotthardson (1955). Since PPLO and gonococci frequently occur together, it is likely that some of the patients with gonococcal salpingitis were also infected with PPLO. The varying clinical response to penicillin of the patients with gonococcal salpingitis also bears this out; the gonococci were cleared, but the salpingitis sometimes persisted owing to PPLO infection. The clinical failure of some patients to react favourably to antibiotics to which PPLO are sensitive need not rule out the presence of this organism as it has been shown that the regression of subcutaneous abscesses caused by PPLO in rats was not hastened by the administration of very large doses of oxytetracycline (Klieneberger-Nobel, unpublished observation). The failure of the oxytetracycline to penetrate the encapsulated abscess would explain why it was ineffective and this probably applies also to the salpingitis which did not respond to antibiotics.

In all except one of the patients with positive sera, antibody was present in low titre, but this is consistent with previous findings. Titres of antibody higher than 12 were found in only five out of 27 patients examined by Melén and Gotthardson (1955). No clinical information was given regarding the severity of the disease in these five patients as compared with the rest, but the authors considered that patients might develop high titres if infections were deeply localized in the adnexa. The highest titres recorded by Stokes (1955) were found in patients who were severely ill and had high temperatures; in contrast, her patient with a clinically mild infection had a low titre of 24 to 48. The low titres of PPLO antibody so frequently found in patients with genital tract diseases may be due to the localized nature of many such infections. It has been observed that rats with a generalized PPLO infection develop higher antibody titres than those with a localized lung infection (Klieneberger-Nobel, 1960; Lemcke, 1961).

In the present series there was a significantly higher proportion of positive tests in patients with a clinically severe infection than in those with a mild infection, although the level of the serum titre was not related to the severity of the case. The high titre of 640 occurred in a patient with severe non-gonococcal salpingitis of 2 months' duration, but there were equally severe cases which were tested at the same stage of the disease and gave only low titres.

The failure to find antibody against the so-called "human genital type 2" strain PG27 in any of 29 sera

tested suggests that the positive reactions between human sera and antigens of the human genital type 1 PPLO are specific. This is consistent with the observations on the specificity of CFT in various PPLO animal diseases (Klieneberger-Nobel, 1960; Lemcke, 1961). In these animals specific antibody against PPLO was found in the blood, and at the same time PPLO were isolated from diseased organs or lesions.

The proportion of positive complement-fixation tests against PPLO in our group of salpingitis patients, the specificity of the reaction, and the persistence of the same reaction in a series of specimens from the same patient, all suggest that the positive serum reactions observed are significant and indicate that the clinical condition is due to infection with PPLO. Undoubtedly other factors are involved in the aetiology of salpingitis; sometimes the salpingitis may be due to a mixed infection with gonococci and PPLO. Our results suggest that PPLO may be a primary cause of the condition, and may play an active part in the disease.

Summary

The sera of 51 patients with salpingitis were tested for complement-fixating antibodies against PPLO. Half the patients gave a positive reaction, compared with less than 4 per cent. of healthy female blood donors tested in an earlier series in the same laboratory.

There was a higher incidence of positive serum reactions in the clinically severe cases than in the mild ones. Antibody was present in high titre (640) in only one patient. In the rest, titres were of the order 10 to 40. In seventeen patients serial serological tests were performed and it was found that in some a positive titre persisted for many months.

Gonococci were present in 23 patients. The proportion of positive serum reactions against PPLO was similar in the gonococcal and non-gonococcal cases. The response to antibiotics and the occurrence of positive serum titres against PPLO in patients in whom gonococci were present suggest that a PPLO infection co-existed with gonorrhoea in many cases. The finding of positive serum titres in patients with no evidence of gonorrhoea suggests that PPLO can be the sole infective agent.

This work was carried out under the aegis of the Medical Research Council Working Party on Non-Specific Urethritis, with the aid of a grant from the U.S. Public Health Service.

We wish to thank Miss M. A. M. Bigby, Dr. F. J. G. Jefferiss, and Dr. R. R. Willcox for their permission to cite cases under their care.

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Anticorps contre les organismes microbiens ayant la morphologie générale du microbe de la pleuropneumonie des bovidés (P.P.L.O.) chez les malades atteints de salpingite

RÉSUMÉ

Le sérum de 51 malades atteints de salpingite fut examiné pour les anticorps fixant le complément contre les P.P.L.Os. Si la réaction fut positive dans la moitié des cas, elle ne l'avait été que dans 40% des cas pour une série de donneuses de sang en bonne santé examinée au même laboratoire.

L'incidence de réactions positives fut plus élevée dans les cas les plus sévères. Il n'y eut un titre élevé (640:1) que dans un seul cas; les autres titres furent de 10 à 40:1. Chez 17 malades, des tests en série montrèrent un titre positif qui dura pendant plusieurs mois.

Ou constata la gonorrhée chez 23 malades. Les sérums positifs furent aussi fréquents parmi ceux-ci que parmi les autres. La réponse aux antibiotiques et l'incidence de tests positifs chez les malades atteints de gonorrhée indiquent que l'infection avec P.P.L.Os. et celle avec la gonorrhée peuvent exister toutes deux à la fois chez le même sujet. D'autre part les réactions positives chez les malades sans gonorrhée indiquent que l'infection avec P.P.L.Os. peut exister indépendamment.