Diagnosis of *Treponema pallidum* in vitreous samples using real time polymerase chain reaction

We describe a real time polymerase chain reaction (PCR) technique for the detection of treponemal DNA in the vitreous of patients with suspected syphilitic uveitis.

Case 1

A 41 year old white homosexual male presented with a 1 week history of pain, redness, and reduced visual acuity in the right eye. There was a recent history of mouth ulcers and skin rashes involving the left lower limb. Corrected Snellen visual acuities were 6/60 and 6/6 in the right and left eyes respectively. In the right eye there were 4+ cells in the anterior chamber and vitreous. The right optic disc was swollen with patchy retinitis involving the inferior quadrants. The left eye was normal. *Treponema* specific serology tests, total antibody enzyme immunoassay assay (EIA) and *Treponema pallidum* particle agglutination test (TPPA) were strongly positive. Rapid plasma reagin (RPR) titre was 1:512, consistent with active treponemal infection. Subsequent cerebrospinal fluid analysis was also positive for both RPR and TPPA, with the additional finding of lymphocytosis.

PCR evaluation of vitreous for herpes viruses and *Toxoplasma* revealed a positive result for Epsein-Barr virus. An in-house TaqMan probe based real time PCR assay (Applied biosystems, UK) targeting the *Treponema pallidum* repeat protein C (Tpr C) gene detected the presence of the *T pallidum* DNA.[2] The patient's DNA extract was tested in parallel with *T pallidum* Nichols strain Seattle genomic DNA as a positive control and a DNA extract previously tested negative for *T pallidum* as negative control. All samples were tested in duplicate (fig 1). The results confirmed the presence of *T pallidum* DNA in the vitreous and, with serological and CSF evidence, a diagnosis of secondary syphilis with syphilitic panuveitis was confirmed.

Case 2

A 53 year old man presented with a 1 month history of rapidly progressive decrease in vision in the left eye. His visual acuities were 6/6 in the right eye and counting fingers in the left eye. He had a panuveitis in the left eye, with evidence of 4+ cells in the anterior chamber and vitreous. There were multiple fluffy white opacities in the vitreous and

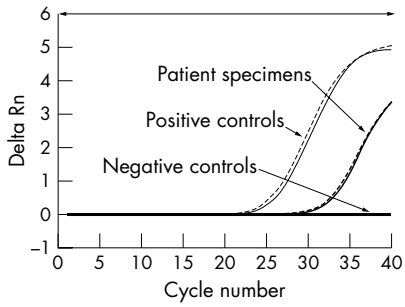

Figure 1 Real time PCR amplifications plot of the vitreous sample of case 1 tested for treponemal DNA in duplicates. The change in normalised reporter signal (Delta Rn) was interpreted with *T pallidum* positive and negative controls.

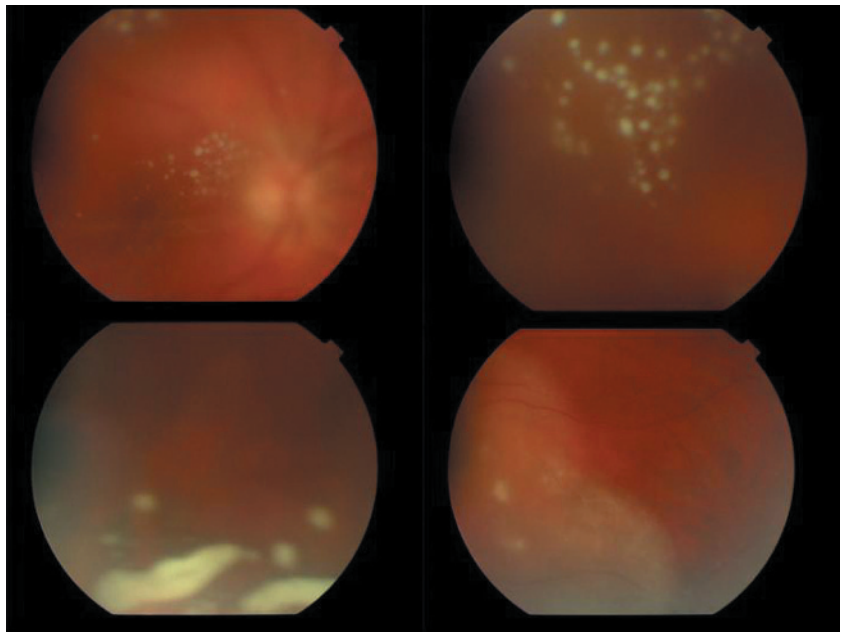

Figure 2 Fundus photographs showing intense vitritis, an unusual presenting sign of acute syphilitic posterior uveitis that resolved completely following a 4 week course of oral doxycyclin therapy.

fundus examination revealed peripheral retinal infiltrates (fig 2). A working diagnosis of acute retinal necrosis was made but vitreous PCR results were negative for herpes viruses and *Toxoplasma*. There was a poor clinical response to systemic valaciclovir therapy and within a 3 week period, he developed multiple vitreous opacities and peripheral retinal infiltrates in the right eye. His treponemal serology was strongly positive and the RPR titre was 1:256, indicating active treponemal infection. Additionally, he was positive for treponemal IgM tested by EIA. Cerebrospinal fluid showed lymphocytosis. PCR analysis of vitreous detected treponemal DNA and a diagnosis of bilateral syphilitic panuveitis was established and appropriate therapy begun.

Comment

Serology is the mainstay of diagnosis of syphilis.[1] However, serological testing cannot identify whether there is active infection in a particular organ. This has led to the development of PCR based techniques, which specifically target difficult diagnostic areas such as neurosyphilis and congenital syphilis.[2–4] To our knowledge, this is the first report of the use of real time PCR to detect *T pallidum* DNA in vitreous samples. While syphilis was adequately diagnosed by serology in both cases, the specific detection of *T pallidum* DNA and exclusion of other infective agents, particularly herpes simplex virus and varicella zoster virus, helped in patient management. This is particularly important in HIV infected patients who may have concurrent infection by more than one agent.[5]

The real time PCR method with a specific fluorescent labelled probe used in our patients is specific for *T pallidum*. However, none of the diagnostic methods, including DNA based tests, can distinguish between syphilis and infection with other pathogenic treponemes, including yaws, pinta, nonvenereal endemic syphilis, or bejel.[4]

As there is only limited experience of using this PCR test on vitreous samples, further studies are required to establish the sensitivity and specificity. Nevertheless, the test can provide confirmation of the diagnosis of ocular disease in patients with positive syphilis serology and adds to the diagnostic evaluation of patients who may have multiple infections.

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Viscogonioplasty in patients with chronic narrow angle glaucoma

Chronic narrow angle glaucoma (CNAG) occurs when the anterior chamber drainage angle progressively narrows with a subsequent rise in intraocular pressure (IOP) and, if this rise is maintained, glaucomatous optic neuropathy. Although there may be a number of reasons for this narrowing, if the peripheral iris remains apposed to the trabecular meshwork for any length of time it is likely that a more permanent adhesion occurs (PAS). Thus even though the underlying cause for the narrowing is removed—for example, pupillary block after peripheral iridectomy (PI) or cataract extraction in lens induced disease, the angle remains closed and the IOP remains damagingly high.

We have previously described a technique for use in acute angle closure glaucoma where the lens induced narrowing is removed by cataract extraction and the PAS broken with viscoelastic. This manoeuvre has been called viscogonioplasty (or VGP).[1] This case series describes the results of this same technique in patients with chronic narrow angle glaucoma

Case series

From April 2002 to March 2005 all patients with CNAG inadequately controlled on conventional therapy were enrolled. The inclusion criteria were as follows:

- Occludable angle confirmed by gonioscopy
- Evidence of uncontrolled IOP (raised IOP, progressive glaucomatous optic neuropathy, and/or visual field progression)
- Previous patent PI.

An occludable angle was defined as the angle in which the posterior (usually pigmented) trabecular meshwork was seen for less than 90° of the angle circumference.[2]

Exclusion criteria included:

- Plateau iris syndrome
- Previous glaucoma surgery (argon laser trabeculoplasty/trabeculectomy)
- Retinal disease
- History of ocular injury
- Other glaucomas.

The duration of increased IOP or synechial angle closure was not one of our inclusion or exclusion criteria. However, all patients in the case series had been diagnosed and treated for at least 3 years. None of the patients had any stigmata of acute angle closure or an acute episode of raised IOP precipitating their initial presentation.

Preoperatively and postoperatively, all patients underwent complete ocular examination The number of glaucoma medications taken by each patient was recorded preoperatively and at the latest clinic visit. The same surgeon performed phacoemulsification and VGP in all the cases. Consent was given by all patients before the operation.

All patients underwent routine phacoemulsification under topical anaesthetic. Following IOL implantation a heavy viscoelastic was used to deepen the anterior chamber and then injected near the angle without touching the trabecular meshwork. No surgical instrument was used to physically break the PAS. Upon completion of VGP the viscoelastic was meticulously removed. Postoperatively, topical steroid and non-steroidal drops were given four times a day in the operated eye for 1 month. All glaucoma medications were discontinued postoperatively and then restarted according to IOP response.

In all, 29 eyes of 18 white patients with poorly controlled narrow angle glaucoma underwent VGP in the study. The mean age of patients was 71.1 years (range 53–95 years). The mean IOP before surgery was 27.4 mm Hg. The patients were followed up for at least 6 months. Mean follow up of patients was 7 months (range 6–24 months).

Following phaco-VGP the mean IOP was reduced from 27.4 mm Hg to 12.2 mm Hg at 6 month review; 6/29 (21%) eyes completed 1 year follow up and mean IOP at 1 year was 11.8 mm Hg. None of the patients needed subsequent trabeculectomy or any other surgical intervention to lower IOP. 25/29 eyes (86%) were controlled without any antiglaucoma therapy. Of the remaining four patients none of them were on more than one topical medication.

Postoperatively gonioscopy showed opening of angle with absence of PAS in all cases.

Table 1 Results of CNAG patients undergoing phacoemulsification and VGP

	IOP (mm Hg)			Postop IOP (mm Hg)			
Case No	IOP at presentation	Immediate preop on treatment	Preop glaucoma medication	2 weeks	3 months	6 months	Postop glaucoma medication
1	34	28	2	17	17	11	0
2	40	27	2	17	18	12	0
3	28	25	4	13	15	10	1
4	48	32	4	9	16	16	1
5	34	20	3	17	12	12	0
6	34	29	3	12	13	13	0
7	42	28	2	14	12	12	0
8	62	40	2	14	18	14	0
9	26	24	2	12	14	11	0
10	30	20	2	17	12	10	0
11	25	22	2	22	20	12	0
12	34	27	3	19	11	11	0
13	31	20	3	17	12	10	0
14	36	30	2	15	16	15	0
15	32	28	2	20	22	18	0
16	42	32	4	12	14	12	0
17	35	28	4	12	13	12	0
18	54	45	2	8	12	12	0
19	43	32	4	18	14	12	0
20	42	20	2	12	16	10	0
21	29	24	3	20	13	13	0
22	36	26	2	12	10	10	0
23	35	29	3	15	15	11	1
24	37	25	3	15	15	12	1
25	36	25	1	18	14	14	0
26	39	30	4	15	18	11	0
27	38	29	4	11	11	12	0
28	30	25	3	10	13	12	0
29	31	25	2	20	19	16	0
Mean IOP (mm Hg)	29.5	27.4		14.9	14.6	12.2	