

Additional Evidence for Chlamydia in Tissues of Patients with Crohn's Disease

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Abstract

Background and aims: Previously, evidence of chlamydia has been reported in Crohn's Disease. The aim of the current work was to seek evidence of chlamydia in resected tissues of patients.

Methods: A specific TaqMan pan-chlamydiae probe was employed in a broad-range Polymerase Chain Reaction (PCR) to seek evidence of various members of the chlamydiae phylum in paraffin-embedded formalin-fixed small bowel tissues from 28 patients.

Results: Chlamydia was identified in 14 of 34 tissue sections from the 28 patients. There was evidence of *Chlamydia suis*, *C. trachomatis*, *Neochlamydia* sp, *Protochlamydia* sp., and *Metachlamydia* sp.

Conclusions: These findings are consistent with earlier studies and an infectious pig model generated by the oral administration of *Chlamydia suis*. It is incumbent upon future studies to seek chlamydia species by PCR as we have done. Resection or biopsy tissues taken early in the course of the disease hold the greatest opportunity to find evidence of chlamydia.

Keywords: Crohn's disease; Dalziel's disease; Chlamydia; Polymerase Chain Reaction (PCR); Lymphatics

employing a broad-range PCR targeting all the members of the Chlamydiae phylum.

Materials and Methods

Tissues were scraped from the slides; there were 34 slides representing resected ilial tissues from 28 patients. DNA extraction was done on a Maxwell® 16 IVD instrument (Promega) with a Maxwell® 16 FFPE plus LEV DNA purification kit (Promega, AS1130) following manufacturer's protocol, elution in 50 µL. A Qubit dsDNA high sensitivity kit (Thermo-Fisher, ref. Q32851) was employed for DNA quantification. Pan-chlamydiae TaqMan real-time quantitative PCR assays were done in duplicate, as described by Lienard et al. [2]. We employed as the forward primer panCh16F2 (5'-CCGCCAACACTGGGACT-3') and as the reverse primer pan-Ch16R2 (5'-GGAGTTAGCCGGTGCTCTTTAC-3') and the probe panCh16S (5'-FAM (6-carboxyfluorescein)-CTACGGGAGGCTGCAGTCGAGAATC-BHQ1 (Black Hole Quencher 1)-3'), which was targeting a fragment of around 207 to 215 bp in the 16S rRNA gene.

Sanger sequencing was done by Microsynth (Balgach, Switzerland) using the specifically designed inner primers panFseq (5'-CCAACACTGGGACTGAGA-3') and panRseq (5'-GCCGGTGCTCTTTAC-3') [2]. Sequences were cleaned and blast was done using Geneious Prime Software (Biomatters, New Zealand), which provided genus or species level identification using the best Blast hit.

Results

Chlamydia were identified in 5 of the 28 cases, as shown in **Table 1**. Due to the short length of the sequence, identification at species level is not accurate and it was not possible to differentiate *C. trachomatis* from *C. suis* in two samples. Similarly, it was not possible to assign at species level, the sequence obtained from representatives of the *Neochlamydia*, *Parachlamydia* and *Metachlamydia* lineages (all part of the *Parachlamydiaceae* family).

Description

Earlier in 2016, we recognized evidence of chlamydia in a few patients with Crohn's disease [1]. Few samples taken from these subjects were positive by PCR and/or by immunohistochemistry and few cases were documented by serologic response. In 2021, we found that we had multiple boxes of unstained tissue cuts mounted on silanized slides from 20 to 30 cases. At the Zurich and Lausanne Laboratories, multiple such unstained four-micron cuts were assembled for a new PCR attempt, this time

Table 1: Patient data and characteristics of the chlamydia.

Sample	Age (yr)	Sex	Duration* (mo)	Best matched organism	%Identity	Seq. length
357	17	F	12	<i>Chlamydia suis</i> OR <i>Chlamydia trachomatis</i>	99.4	206
357	-	-	-	<i>Neochlamydia hartmannellae</i> strain A1Hsp	92.5	203
354	18	M	12	<i>Neochlamydia</i> sp.	97.5	140
354	-	-	-	<i>Protochlamydia naegleriophila</i>	94.7	107
199506	23	F	5	<i>Candidatus Metachlamydia lacustris</i> strain CHS	97	204
211	26	F	6	<i>Chlamydia suis</i> OR <i>Chlamydia trachomatis</i>	99.1	205
86695	26	M	36	<i>Chlamydia trachomatis</i>	99.3	206

Note: *Duration of disease from onset to surgical resection.

Discussion

Earlier we reported the presence of chlamydial DNA in 3 of 33 patients [1]. Here we report results obtained with a pan-Chlamydiae Taq Man quantitative PCR assay done in a separate laboratory. One of the positive cases, number 199506, was already found to contain chlamydial DNA in that earlier study and again here. Tissues from that patient were positive by immunohistochemistry as well [1]. The other four positives had either not been done (n=1) or were negative (n=3) in the earlier study.

Evidence for chlamydia in the resection tissues of CD patients continues to be found, even in patients who have had the disease for many years prior to intestinal resection. The fact that the TaqMan assay use of fluorescent probe and read-out is done with closed tubes, a vertical or horizontal PCR contamination with amplicons is avoided. Moreover, such results are unlikely to be due to PCR contaminants since a false positive occurs in our hands with this closed-tube technology in less than 1/1000 samples tested and the fact that one of the 5 positive cases was previously positive by immunohistochemistry and using a PCR done in another laboratory.

Noteworthy, this TaqMan PCR is highly sensitive with as few as 10 copies/reaction cupule, thanks to the short sequence amplified (about 200 bp) and to the use of fluorescent probes and lasers to detect the signal. However, due to the short sequence obtained and the high degree of 16S rRNA gene sequence conservation, there is only one or two base pairs

difference between distinct species of the genus chlamydia. Therefore, we cannot conclude whether the chlamydial DNA is from *Chlamydia suis* or from *Chlamydia trachomatis*. This finding is nevertheless of high interest, given the potential of these well recognized pathogenic bacteria to play a role in chronic lymphocytic and granulomatous disease [3,4].

We have shown that *Chlamydia suis*, under experimental conditions, crosses the intestinal barrier and then produces necrosis in mucosal and submucosal lymphatic endothelium [5], where the early lesion of Crohn's disease occurs [6]. All species of chlamydia of farm animal origin have zoonotic potential [7-9]. We need to continue to use PCR with pan-chlamydia probes on the interstitial tissues of patients with early Crohn's disease. Endoscopic biopsies may be inadequate because of the superficiality of the sampling [10], but wedge biopsies may be important to uncover the possible implication of chlamydia in the pathogenesis of Crohn's. Although here we report findings obtained from aged specimens, undoubtedly, specimens obtained at the onset of disease offer the greatest opportunity to find evidence of chlamydia.

Conclusion

These findings are consistent with earlier studies and an infectious pig model generated by the oral administration of *Chlamydia suis*. It is incumbent upon future studies to seek chlamydia species by PCR as we have done. Resection or biopsy

tissues taken early in the course of the disease hold the greatest opportunity to find evidence of chlamydia.

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