Failure to Detect *Chlamydia pneumoniae* in Major Arteries of 93 Patients with Atherosclerosis

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Background

- **Seroepidemiological studies have indicated a possible association between chronic *Chlamydia pneumoniae* (*Cp*) infection and atherosclerosis**
- **Several studies, using different diagnostic methods have demonstrated *Cp* or its components in atherosclerotic lesions**
- **These findings have not been confirmed by all researchers**

Aims of the Study

- To detect *Cp* in surgical specimens from major arteries in patients with atherosclerosis

Patients

- **From Sep 1, 1999 to Feb 28, 2000**
- **Admitted to cardiovascular surgery for coronary bypass or vascular surgery for carotid endarterectomy at RMC- Beilinson Campus**
- **Study approved by ethics committee.**
  Patients signed informed consent
- **Data collected by questionnaire on demographics, underlying diseases, risk factors for atherosclerosis and antibiotic usage**

Serologies

- **Blood specimen collected before surgery**
- **Cp IgG, IgA and IgM antibodies tested by microimmunofluorescence test (MIF) and enzyme-linked immunosorbent assay (ELISA)**
- **MIF- MRL Diagnostics, USA. IgM sera screened at 1:10 IgA and IgG 1:16**
- **ELISA- Sero CP-TM – Savyon Diagnostics Ltd, Israel (Cut-off index 1.1)**

Tissue Specimen Collection

- **Coronary bypass- 2 to 4 punch specimens from the aortic wall**
- **Carotid endarterectomy- atheromatous plaques**
- **In the operating room, specimens placed immediately in Chlamydia media transport (sucrose-phosphate-glutamic acid, SPG)**
- **Specimens delivered to the lab within 15-20 minutes**
- **Homogenized and resuspended in SPG andd stored at –70 degrees for PCR**

PCR

- In two different laboratories
- DNA extraction using the ViralXpess (Chemicon)
- PCR
  - RMC- Light Diagnostics OligoDetect (Chemicon)
  - Immunosciences Lab- assay as described by Campbell
- Primers
  - Sense: 5'TCA.ATC.AGC.CAT.TCA.TAA.CA-3'
  - Antisense: 5'GGG.ATT.GTA.GTA.TTT.CTC.TC-3'

Culture

- **Resuspended, homogenized specimens inoculated onto shell vials containing monolayers of cycloheximide treated HTp-2 cells**
- **Incubated at 37 degrees in 5 % CO2 for 3-4 days**
- **Each specimen incubated onto 4 shell vials**
  - **Giemsa**
  - ***C pneumoniae* specific fluoresceine conjugated monoclonal antibody**
  - **2 vials used for subculture**
- ***C pneumoniae* TW-183 used as control in each experiment**

Results- Characteristics of Patients

Mean age (range)		67 (47-83)
M:F ratio		2.4
Type of surgery		
Bypass	61	
Endarterectomy	32	
Smoking		31%
Hypertension		63%
Diabetes mellitus		43%
Hyperlipidemia		58%
Antibiotics		16%

Results-
Chlamydia Serology in 83 Patients (%)

	ELISA	MIF	Both
IgM	1	1	1
IgG	55	67	47
IgA	35	27	17

Results-
PCR and Cultures

- Cultures for Cp were negative in all specimens
- All PCR tests performed by the two methods in two different laboratories gave negative results

Comments

- Sampling error problems (blind specimens from the aorta)
- PCR techhniques for identification of Cp may be hindered by difficulty of DNA extraction from atheromatous material and by the presence of PCR inhibitors
- Immunocytochemistry appears to be more sensitive

Previous Studies on PCR

- 19 studies (1993-2001)
- 5-238 specimens per study
- Overall 949 specimens
- Positive from 0 to 100 %
- Overall 247/949 (26%) positive

Previous Studies on Culture for *Chlamydia*

- 10 studies (1993-2001)
- 3 to 58 specimens per study
- Overall 296 specimens
- Positive from 0 to 16 %
- Overall 7/296 (0.02%) positive

Conclusions

- In our study population, we found no evidence that *Chlamydia pneumoniae* exists within atheromas in carotid arteries nor in samples obtained from the aortic wall of patients with atherosclerosis

Collaborators

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