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 $C_p$ 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 and 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’ 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 (%)

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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 techniques 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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