

## Increased Chlamydia Pneumoniae IgG after Coronary Artery Bypass Surgery

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### Abstract

**Aim:** The increasing body of evidence influences the possible association of Chlamydia pneumoniae infection with pathogenesis of atherosclerosis. The influence of coronary artery surgery on the disease activity is unclear. We aimed to determine the seroprevalence of *C. pneumoniae* before and after the coronary artery bypass grafting (CABG) surgery.

**Methodology:** One hundred and seventy eight patients diagnosed with coronary artery disease undergoing surgery were involved in the study. *C. pneumoniae* IgG and IgM seroprevalence were examined using ELISA method both the day before and 30 days after the surgery.

**Results:** IgG and IgM seroprevalence for *C. pneumoniae* were found to be 63.45% and 2.8% before the surgery. While there was significant increase on IgG (69.66%,  $p=0.001$ ), increase was not significant on IgM values (4.49%,  $p=0.250$ ).

**Conclusion:** Our study showed that CABG surgery induced activation of chronic *C. pneumoniae* infection with surgical stress-induced immunological changes. Other possible factors, such as blood transfusion, are likely to be involved in this activation. Antimicrobial therapy during the operative period should be considered in patients who are serologically positive for *C. pneumoniae* after an operation.

**Keywords:** Coronary Artery Disease; Chlamydia Pneumoniae; Serology; Prevalence; Cardiovascular surgery

### Background

Coronary artery disease is one of the most common causes of morbidity and mortality in the western community [1]. The progress of the disease is slow that makes difficult to define underlying etiopathogenesis. Hyperlipidemia, hypercholesterolemia, diabetes, cigarette smoking, genetic predisposition, and hypertension are accepted as common risk factors that determines only 50-60% of the patients. Studies performed in the recent years indicated the important role of the chronic infections in the progression of the disease [2,3].

*C. pneumoniae*, is an obligate intracellular microorganism that cause of upper respiratory tract infection. It has been considered as one of the infectious etiologies of atherosclerosis that first demonstrated by Saikku et al. [4]. The microorganism first appears in the lower respiratory tract and infected alveolar macrophages that transmigrate from the epithelium enter to the circulation and reach atherosclerotic plaque. *C. pneumoniae* may be capable of infecting the variety of cells including macrophages, vascular endothelial and smooth muscles. The presence of organism on atherosclerotic plaque has been shown by various techniques, including polymerase chain reaction, immunohistochemistry, and electron microscopy [5,6].

Although the mechanisms are not clarified, the cell-mediated immunity is depressed especially with on pump cardiopulmonary bypass surgery [2,7]. Cardiac surgery has been also demonstrated to

induce inflammatory cytokines and immune suppression [8]. Corticosteroid use during surgery further increased this effect [9]. Therefore, the possible change on serologic profile of disease is possible for the intracellular microorganism interacting with cell mediated immunity during surgery for the coronary artery disease that may be induced by various mechanisms [2]. The objective of the present study was to investigate the serologic profile of *C. pneumoniae* in the patients undergoing surgery for coronary artery bypass grafting (CABG) before, and after the surgery.

### Methods

The study was performed under approval of Institutional Review Board and informed consents were obtained from each patient in Ministry of Health Yıldırım Beyazıt Hospital, Department of Cardiovascular Surgery March 2006 to October 2010. One hundred and seventy eight patients diagnosed with coronary artery disease undergoing CABG surgery were consecutively included to the study. As a part of their routine biochemical screening, venous blood samples withdrawn from patients before and 30<sup>th</sup> day after the operation. To focus on just coronary artery disease and discuss it on this field, the patients with rheumatoid valve or congenital heart disease, renal insufficiency, any type of malignancy, myocardial infarction within the past three months and unstable angina pectoris were excluded from the study. These patents were also rare to get them in to data. Patients suffered upper respiratory infection within 30 days after operation were excluded from the study. On-pump coronary surgery was performed under standard opioid-based anesthetic technique

including fentanyl, isoflurane, vecuronium bromide, using internal mammary artery or saphene vein graft. Prophylactic antimicrobial therapy (cefazolin 1g i.v.) was given all patients during the preoperative period.

## ELISA

Serum samples from the blood were separated and stored at minus 70° until analyses. Investigator performing the ELISA was blinded about the origin of the samples including study or control patients. Commercially available kits (Euroimmun, Lübeck, Germany) that qualitatively screening the IgG and IgM class antibodies against *C. pneumoniae* were used. The serum samples were thawed at room temperature and diluted at 1/100 for determining IgG and 1/10 for IgM, respectively. Controls and standards were performed using double-hole technique. Samples were remained about 20 min before investigating *C. pneumoniae* IgM for excluding the rheumatoid factors from the samples, and care was undertaken to obtain from the upper portions before distributing to the holes. All serum samples were incubated at room temperature for 30 min. All holes were rinsed with 300 µL solution using automatic machine (BIO-TEK ELx50). A specific enzyme conjugate 100 µL was added to the each hole and incubated 30 min in room temperature. Samples were rinsed again with 300 µL solution for three times and 100 µL chromogen substrate was added and incubated in a dark at room temperature for 15 min. A stop solution 100 µL was added to the samples after incubation period and optic densities (At 450 nm, ref 650 nm) were determined using ELISA reader (SEAC Radim). The units corresponds the optic densities which were determined.

Results from *C. pneumoniae* IgG under 16 RU/mL were determined as negative and values exceeding 22 RU/mL were accepted as positive, and samples were reevaluated between these values. A cut-off value was determined for IgM and upper and lower values were accepted as positive and negative respectively. The values near the limit were again reevaluated.

## Statistics

Data were analyzed using McNemar and Pearsons x2 tests using a package program (SPSS for Windows version 15.0, SPSS Inc. Chicago, IL, USA). The relative risk values were determined after obtaining 95% confidence intervals. A p value was considered as 0.05 for statistical significance.

## Results

One hundred and seventy eight patients (138 male, and 43 female), aged mean 56.24 years (ranging 24-74) were included to the study. The distribution of age and gender are shown in Table 1. All patients were undergone to the CABG using a classical technique. One coronary bypass or grafting was performed in 67 patients, two bypass required in 39, three bypass was necessary in 39 patients, and left ventricular aneurysm repair was performed in 29 patients. 96 of 135 male patients (71.11%) and 4 of 43 female patients (9.3%) were smoking one pack year or more. Under 30 years of age no male or female patients were smoking.

| Age Interval | Male | Female | All | %   |
|--------------|------|--------|-----|-----|
| 15-19        | 0    | 0      | 0   | 0   |
| 20-24        | 1    | 1      | 2   | 1.1 |

|        |     |    |     |      |
|--------|-----|----|-----|------|
| 25-29  | 0   | 0  | 0   | 0    |
| 30-34  | 1   | 1  | 2   | 1.1  |
| 35-39  | 7   | 1  | 8   | 4.5  |
| 40-44  | 9   | 3  | 12  | 6.7  |
| 45-49  | 13  | 6  | 19  | 10.7 |
| 50-54  | 24  | 6  | 30  | 16.9 |
| 55-59  | 31  | 6  | 37  | 20.8 |
| 60-60+ | 49  | 19 | 68  | 38.2 |
| Total  | 135 | 43 | 178 | 100  |

**Table 1:** Distribution of age and gender.

There was significant difference on seropositivity for IgG anticor against *C. pneumoniae* between preoperative (63.45%) and postoperative values (69.66%, p=0.001). On the other hand, a significant difference was not determined for IgM values including before (2.8%) and after (4.49%, p=0.250) the surgery. Age related changes on *C. pneumoniae* IgG and IgM values are detailed on Table 2.

| Surgery | Before |       |     |      | After |      |     |      |
|---------|--------|-------|-----|------|-------|------|-----|------|
|         | IgG    |       | IgM |      | IgG   |      | IgM |      |
|         | N      | %     | N   | %    | N     | %    | N   | %    |
| 15-19   | 0      | 0     | 0   | 0    | 0     | 0    | 0   | 0    |
| 20-24   | 2      | 1.1   | 0   | 0    | 2     | 1.1  | 0   | 0    |
| 25-29   | 0      | 0     | 0   | 0    | 0     | 0    | 0   | 0    |
| 30-34   | 1      | 0.56  | 0   | 0    | 1     | 0.56 | 0   | 0    |
| 35-39   | 4      | 2.24  | 0   | 0    | 4     | 2.24 | 0   | 0    |
| 40-44   | 11     | 6.18  | 0   | 0    | 12    | 6.74 | 1   | 0.56 |
| 45-49   | 13     | 7.3   | 1   | 0.56 | 15    | 8.42 | 2   | 1.1  |
| 50-54   | 16     | 8.99  | 1   | 0.56 | 18    | 10.1 | 1   | 0.56 |
| 55-59   | 24     | 13.48 | 1   | 0.56 | 25    | 14.1 | 1   | 0.56 |
| 60-60+  | 42     | 23.6  | 2   | 1.1  | 47    | 26.4 | 3   | 1.68 |
| Total   | 113    | 63.45 | 5   | 2.8  | 124   | 69.7 | 8   | 4.49 |

**Table 2:** Age-related change on *C. pneumoniae* IgG and IgM seropositivity.

## Discussion

Atherosclerosis is the most important pathological process involved in coronary heart disease. Coronary artery disease is the most likely infectious etiology for patients undergoing surgery for CABG. The association between *C. pneumoniae* and coronary artery disease is well documented. However, little is known regarding *C. pneumoniae* seropositivity in patients undergoing CABG. *C. pneumoniae* cellular structures and genomic DNA segments have been directly observed in atherosclerotic vessels [9-11]. A recent study by Gürkan et al. found a

significant correlation between increased aortic intimal thickness and *C. pneumoniae* seropositivity, although the exact cause was not determined [12]. The seroprevalence of *C. pneumoniae* is increased with advancing age, which could explain the higher incidence of *C. pneumoniae* in our study population. An even higher rate of *C. pneumoniae* was obtained in a recent study that investigated atherosclerotic patients from the same region. However, IgM values in this previous study are comparable with our results [13]. Our study showed a significant increase in *C. pneumoniae* IgG after CABG surgery compared with before CABG surgery, and a slight change in IgM values was observed after CABG surgery. Although this percentage change was small, it clearly demonstrates recent activity of the disease and the opportunistic nature of the microorganism. IgM is the first immunoglobulin to appear against *C. pneumoniae* that should be detected after 3 weeks. However, 6 to 8 weeks is required for an IgG response. To the best of our knowledge, this is the first report to find an increase in disease activity in the same population [2,14].

Seropositivity may increase with contamination of blood or blood products with *C. pneumoniae* in the perioperative period. Approximately five units of blood or blood products were provided to every patient during CABG surgery during priming and on maintenance. Therefore, in the postoperative period, the seropositivity of *C. pneumoniae* may be increased, which may explain the results in our study. Donors can only give one unit in the limited time period. Therefore, five different persons' blood had to be provided (five units) to the same patient during surgery. *C. pneumoniae* seropositive transfusion can be performed in patients from donors that may result in a five-fold increase in seropositivity. In addition, if donors in our study had acute *C. pneumoniae* infection while giving blood to patients, this could also explain our finding of increased *C. pneumoniae* IgM seropositivity. Birkebaek et al. found IgG antibodies to *C. pneumoniae* in 42 (40%) healthy blood donors and IgM antibodies to *C. pneumoniae* in one (1%) healthy blood donor in their study [14]. Gencay et al. found IgG antibodies to *C. pneumoniae* in 64.3% of healthy adults, and this result may vary by location and time [15]. In a recent cohort study, antimicrobial therapies that were administered to Chlamydia-positive patients did not lead to symptomatic improvement in peripheral vascular disease [16]. Community-based eradication programs with antimicrobial therapy might not be effective in the dormant state of the microorganism. The presence of *C. pneumoniae* in atherosclerotic plaques, but its rare occurrence in the healthy vasculature, indicates that this microorganism might spread through dissemination from a primary infectious site with immune cells, namely T lymphocytes or monocytes [17]. This theory further supports the requirement of depletion of T cell function for activation and/or dissemination. Because of diversity in the population who are aged 15-35 years, specific age-related changes concerning *C. pneumoniae* seropositivity are difficult to determine.

Major surgery is able to induce immune suppression in the perioperative period by producing surgical stress, trauma, and preceding neuroendocrine responses, which affect T cell function and inflammatory mediators [18]. Anesthetics, including several intravenous agents, volatile anesthetics, and opioid use, as well as hypothermia, have also been demonstrated to contribute to immune suppression with decreasing T cell activity. The majority of these factors are mandatory in this type of operation [16]. In an in vitro study, opioids specifically inhibited T cell growth factor, other transcript activators, and T cell receptor-activated calcium flux in human T lymphocytes [2]. Whenever blood received from donors is

indicated during surgery, administration to the patient may result in alterations in seropositivity.

The reasons for *C. pneumoniae* seroconversion, especially in patients undergoing CABG surgery, warrant further investigation. We speculate that increased inflammatory reactions or stunned immunity due to surgery may be major signals for bacterial reactivation. This requires continuous monitoring of the host's immune status by the microorganism that establishes its insidious nature, even in the dormant state. Heat shock proteins, which are increased in stressful circumstances, have a close homology with *C. pneumoniae*. Heat shock protein-60 may also play a triggering role to activate this disease [18]. This may explain the diversity of the disease course or activity of *C. pneumoniae* between patients. Brunham and Rekart hypothesized that immunity is arrested by large-scale antimicrobial treatment to explain the increased rate of re-infection [16]. Another study showed *C. pneumoniae* antibodies in 79.7% of patients and 43.2% of controls. Additionally, elevated *C. pneumoniae* antibodies were also reported in male survivors of myocardial infarction [17]. We did not include a control group in our study because the primary aim of our study was to investigate the serological profile of *C. pneumoniae* in patients before and after surgery for CABG. In a study from our region, the control group had similar standards and there was a lower ratio (34%) of *C. pneumoniae* IgG seropositivity than in patients [18]. *C. pneumoniae* infection, which is correlated with a chronic inflammatory response, can lead to atherosclerosis and other long-term clinical complications, such as myocardial infarction and stroke [17]. To prevent infectious complications, our clinical routine includes short-term, broad-spectrum antibiotherapy. Eradication of *C. pneumoniae* requires agent-specific antibiotic (azithromycin) treatment for at least 6 weeks [19]. Previous studies have shown that short-term therapy has no efficacy on progression of *C. pneumoniae*-induced atherosclerosis [20].

Our clinic is a referral center for nearby cities and constitutes the majority of patients in our surgical population. However, patients were reluctant to be re-admitted for follow-up cardiac examinations, which were performed locally by cardiologists. Patients who were neglected or lost are possible reasons for loss of follow-up. Therefore, long-term follow-up was not possible, and is a limitation of the present study.

## Conclusion

Our results suggest that coronary surgery increases *C. pneumoniae* IgG, and to a lesser extent, IgM seroprevalence. Increased *C. pneumoniae* IgG and IgM after CABG surgery not only depends on immunological mechanisms, but could also increase by blood transfusion from various donors. Therefore, antimicrobial therapy must be provided to all patients who are seropositive for *C. pneumoniae* at the postoperative period. Monitoring of increased seropositivity of *C. pneumoniae* IgM and IgG before and after CABG surgery could be an indicator for developing infection from blood donors. Prophylactic antibiotic therapy should be added to therapy for these patients to decrease their disease activity.

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