# Immunological investigation of the cellular and molecular composition of pericardial fluid in open heart surgery patients

**Ph.D. Thesis**

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# SUMMARY

Although pericardial fluid (PF) is removed from the pericardial sac during open heart surgery, it is not routinely investigated. Composition of PF may reveal immunological processes that play a central role in the course of myocardial infarction, atherosclerosis, and calcific aortic stenosis.

We investigated lymphocyte populations, markers of immune activation in PF, and blood samples of patients undergoing CABG operation, and then compared them to those who had aortic valve (AVR) surgery. We also detected natural anti-CS IgG/M autoantibody levels in plasma and PF of cardiac surgery patients and investigated their relationship with cardiovascular disease-associated bacterial pathogens using enzyme-linked immunosorbent assay (ELISA) and Luminex methods.

The PF contained significantly higher amounts of activated lymphocyte subpopulations ($CD3^+HLA^+$ cells, $CD8^+$ $HLA^+$ lymphocytes) and Tmemo cells, than blood. CABG patients showed significantly elevated amounts of the activated $CD4^+$ helper T cells and $CD4^+$ regulatory T-lymphocytes in both PF and blood compared to the aortic valve replacement group. INKT cells represented the only regulatory lymphocyte population reaching significantly higher concentration in PF than in blood. MCP1 serum level was markedly elevated in CABG group.

The total Ig subclass levels were four to eight times lower in PF than in plasma, but the natural anti-CS IgM autoantibodies showed a relative increase in PF. *Mycoplasma pneumoniae* antibody-positive patients had significantly higher anti-CS IgM levels. In CABG patients we found a correlation between anti-CS IgG levels and *M. pneumoniae, Chlamydia pneumoniae,* and *Borrelia burgdorferi* antibody titers.

Hopefully, our data contributes to future PF analyses that will change and broaden the scientific and clinical importance of the PF.

# INTRODUCTION

## Pericardial fluid is a relevant specimen to investigate cardiac pathology

Pericardial fluid (PF) is a 15-50ml, clear, pale-yellow fluid located in the pericardial space between the parietal and visceral layers of the pericardium. PF is mainly an ultrafiltrate of plasma that includes some overflow of myocardial interstitial fluid as well as myocardial lymph drainage. Therefore, PF reflects the composition of cardiac interstitium, though only scant literature is available on the physiological composition of it. In spite of the possible diagnostic and clinical relevance of the PF analysis, it is not investigated routinely; on the other hand, it is considered to be a reliable specimen due to its anatomical isolation and proximity to the heart. Accumulating evidence shows that PF is a stable biofluid with low clearance rates that accumulates heart-derived factors released from regions as deep as the myocardium. PF composition may well influence the regeneration process following open heart surgery; moreover, PF is also proven to be a suitable vehicle for mesenchymal stem cells. Immunomodulatory therapies can have the potential to limit the infarct volume and accelerate repair. Hence, the exploration of the diagnostic, prognostic, and therapeutic properties of the pericardial space deserve more attention.

## Coronary artery bypass graft (CABG) – and aortic valve repair/replacement (AVR)surgery

CABG surgery is the surgical revascularization of the heart, bypassing the occluded or stenotic coronary arteries with one or more autologous vessel/s. CAD remains the most common pathology with which cardiologists and cardiac surgeons are faced. CABG is performed in patients with significant left main coronary artery stenosis or left main equivalent two or three vessels disease with proximal LAD stenosis. Significance here is defined as having greater than or equal to 70% stenosis

Age-related degenerative calcific aortic stenosis (CAS) is currently the most common cause of acquired aortic stenosis (AS) in adults and the most frequent reason for aortic valve replacement (AVR) in patients with AS, while rheumatic AS represents the least common form. Calcified bicuspid aortic valve represents the most common form of congenital AS. Critical obstruction to the left ventricular outflow is reached

with (1) an increase in peak systolic pressure gradient of greater than 40 mm Hg in the presence of normal cardiac output, and (2) a decrease in effective aortic orifice area of less than 0.5 $cm^2/m^2$ of body surface area (usually 0.8 $cm^2$). Aortic valve replacement is indicated in patients with symptomatic AS.

Aortic regurgitation (AR) is a diastolic reflux of blood from the aorta into the left ventricle owing to the failure of coaptation of the valve leaflets during diastole. AR has numerous causes that can be grouped according to the structural components of the valve affected. Most commonly, aortic valvular insufficiency is seen in combination with AS.

## About the immune system

The innate immune system responds rapidly to infectious agents, whereas the adaptive response requires cell division and differentiation of effector cells. The role of lymphocytes is mediating mainly adaptive immunity. The main populations of circulating lymphocytes in the peripheral blood originate from the common lymphoid precursor, and are called T-, B, and natural killer (NK) cells. In circulation they are mostly present in naive form (mature cells that have never encountered foreign antigens). Naive lymphocytes are activated by antigens to proliferate and differentiate into effector and memory cells. Memory cells may survive in a functionally quiescent or slowly cycling state for years, even lifelong.

B lymphocytes are the only cells capable of producing immunoglobulins (antibodies), thus functioning as the mediators of humoral immunity. Antibodies or immunoglobulins (Ig) are circulating proteins that are produced in response to exposure to foreign structures known as antigens. Memory B lymphocytes may express certain classes (isotypes) of membrane Ig, such as IgG, IgE, or IgA, as a result of isotype switching, whereas naive B cells express only IgM and IgD.

The circulation of healthy individuals contains antibodies that react with self and non-self-antigens, and are generated without external antigen stimulation by B1 cells. These IgM and IgG immunoglobulins considered natural autoantibodies. These autoantibodies serve as scavengers of damaged molecules and cell and have been implicated in the control of inflammation and autoimmune diseases

The major lymphocyte group consists of T lymphocytes, they recognize peptides derived from foreign proteins that are bound to antigen presenting cells. T lymphocytes do not produce antibody molecules.

Cytokines are a large group of secreted proteins with diverse structures and functions that regulate and coordinate many activities of the cells of innate and adaptive immunity. All cells of the immune system secrete at least some cytokines and express specific signaling receptors for several cytokines. The large subset of structurally-related cytokines that regulate cell migration and movement are called chemokines.

## Role of the immune system in cardiovascular diseases and in the pericardial fluid

MI causes sterile inflammation, which is characterized by the recruitment and activation of immune cells of the innate and adaptive immune systems. There is strong evidence that T lymphocytes play a central role in the pathogenesis of cardiac disease, either by direct cytotoxicity, by enhancing the inflammatory functions of other cells, or by helping B cells produce pathogenic antibodies. $CD4^+$ helper T ($T_H$) -, regulatory T- (Treg), natural killer T (NKT) cells, γ/δ T and iNKT cell populations were found to be crucial in the course of cardiovascular diseases including ACS and AMI. However, there is currently a lack of studies that focus on human samples and PF. MI also reactivates an embryonic program in the epicardium, and factors in the PF could exert paracrine actions on certain epicardial cells.

Studies suggest that valvular calcification in CAS is actively regulated and has inflammatory features such as $CD4^+$ and $CD8^+$ T cells that infiltrate the aortic valve. Investigations have shown a clonal expansion of T cells in mineralized aortic valves. A subset of memory T cells is activated in patients with CAS. Immunohistological analyses of explanted mineralized aortic valves showed that $CD8^+$ cells were present at the proximity of mineralized nodules.

In subjects with myocardial ischemia several biomarkers are detectable in PF before they reach measurable concentration in circulation. Numerous heart diseases are associated with altered blood levels of markers and cytokines such as soluble CD40 ligand (sCD40L), tissue plasminogen activator (tPA), monocyte chemoattractant

protein-1 (MCP-1), interleukin-6 (IL-6), interleukin-8 (IL-8), and soluble P-selectin (sPsel).

There is no information available on the presence and function of the natural immune system (an intermediate between the innate and adaptive immune systems) in the PF. Based on previous studies, including those from our laboratory, it is known that citrate synthase (CS), a highly conserved mitochondrial inner membrane enzyme, is an autoantigen recognized by the natural immune system. We have documented previously that anti-CS IgM natural antibodies are continuously present in plasma of healthy controls and that anti-CS IgG levels increase in patients with autoimmune diseases and heart transplantation; however, no data has been published regarding the anti-CS antibodies in PF. Heart muscle cells contain the highest number of mitochondria in body tissues, and mitochondrial enzymes can become released in different cardiovascular diseases. Mitochondrial dysfunction is associated with the development of numerous cardiac diseases such as atherosclerosis, ischemia-reperfusion, injury, hypertension, diabetes, cardiac hypertrophy and heart failure, due to the uncontrolled production of reactive oxygen species.

Infection-related antibodies have also been found to have a potential role in the development of atherosclerosis, including *Chlamydia pneumoniae, Mycoplasma pneumoniae, Borrelia burgdorferi, Helicobacter pylori,* and *Yersinia enterocolitica*.

## AIMS

We hypothesized the existence of a unique immunologic milieu within the PF with its dedicated lymphocyte pool that is reactive to heart-related conditions.

1. Phenotype-analysis of lymphocyte subpopulations, found in the pericardial cavity, was compared to that in the serum samples within individuals who underwent CABG operation (groupA) and aortic valve replacement surgery group (groupB) as a control.
2. Quantitative measurements of cardiovascular cytokines and markers in PF, which were compared to that in the serum samples within the above-mentioned patient groups.

3. The other goal was to determine whether natural autoantibodies to mitochondrial CS enzyme and antibodies to certain bacteria related to atherosclerosis were present in the plasma and PF of cardiac surgery patients.
4. We also aimed to investigate the relationship of the anti-CS antibodies to anti-bacterial antibodies and to the total immunoglobulin class levels in plasma and PF in patients with aortic valve replacement and CABG with or without previous MI.

## MATERIALS AND METHODS

### Patients

Patients admitted for elective open heart surgery were recruited into our study. Individuals were divided into two groups: CABG surgery (GroupA, n=24), and AVR (GroupB, n=12). GroupA had isolated CAD; GroupB had CAS without CAD. For the evaluation of natural autoantibodies we also compared patients within the CABG group: patients with previous MI, and individuals who had no previous MI. The preoperative lab parameters were in normal range (CRP, WBC, ESR included). Exclusion criteria were: type1 diabetes mellitus, any kind of neoplasia, hepatitis, HIV, autoimmune disorders, any conditions treated by plasmapheresis, and any indication for combined heart surgery.

### Pericardial fluid and blood samples

PF samples were drawn with careful, bloodless preparation of the pericardium under the same well-determined conditions. At least 3ml of PF was obtained with gentle suction into heparinized tubes preceding the administration of heparin into the systemic circulation. Simultaneously 10 ml peripheral blood was taken from the central venous catheter. Samples were stored on ice until processing.

### Cell preparation for flow cytometry

Lymphocyte subpopulations in blood and PF were analyzed based on surface marker immunofluorescent staining by flow cytometry. 50$\mu$l of blood and 100$\mu$l of $2x10^{5}$ cell/100 $\mu$l in PBS/BSA/azide buffer resuspended PF pellets were processed. The cells were stained with a set of antibodies on ice for 25 minutes. Subsequently, 2ml lysis buffer was added and incubated at room temperature (RT) for 10 minutes to

eliminate the red blood cells. After PBS/BSA/azide washing and centrifugation (1000 rpm, five minutes) the supernatant was removed and the cells were fixed with 300$\mu$l FACS-FIX solution. The samples were kept at 4◦ ºC until flow cytometric detection was performed on a FACSCalibur™ Flow Cytometer. $10^5$ cells were collected from the lymphocyte gate, and the lymphocyte subpopulations were analyzed and expressed as a percentage of total lymphocytes.

**Multiplex Microbead Technique**

We used Human Cardiovascular 6plex FlowCytomix Kit to measure the concentration of sCD40L, IL-8, IL-6, MCP-1, sPsel, tPA in plasma and PF. The samples were measured with a FACSCalibur flow cytometer. Supernatants of PF samples and plasma were stored at -20°C. After thawing, the sample pairs were centrifuged (16000g, five minutes), then mixed with 1000 beads and incubated with 50$\mu$l biotin-conjugate mixture for two hours at room temperature (RT). To create a calibration curve, standards were diluted by subsequent dilution steps in Assay Buffer mixed with the beads, then 50$\mu$l biotin-conjugate mixture was added and the tubes were incubated at RT for two hours. After two washing steps, 50µl Streptavidin-PE was added to the samples. Finally, following two centrifugation steps (200g, five minutes), the beads were resuspended in 500µl Assay Buffer for flow cytometric acquisition and software analysis.

**Measurement of immunoglobulin isotypes**

MILLIPLEX MAP Human Isotyping Kit was used to measure human IgG subclasses (1, 2, 3, and 4), IgM, and IgA in plasma and PF samples (1:16000 and 1:10 dilutions of plasma and PF, respectively). The samples and the Anti-Human Multi-Immunoglobulin Beads were added to the wet Microtiter Filter Plate and incubated for one hour on a plate shaker at RT. After two washing steps, the samples were incubated with anti-human kappa or lambda light chain antibodies. Following the vacuum removal of the fluid, the beads were suspended in Sheath Fluid and the reaction was read on a Luminex 100™ IS instrument.

**Measurement of anti-CS antibodies by enzyme-linked immunosorbent assay**

Ninety-six well polystyrene plates were coated with 5 mg/ ml CS from porcine

heart in 0.1 M bicarbonate buffer, pH 9.6 at 48C overnight. Following the saturation of non-specific binding sites with 0.5% gelatin in phosphate- buffered saline (PBS) (pH 7.3), plasma samples were incubated in duplicate at 1:100 dilution and PF samples at 1:10 dilution in washing buffer (PBS, 0.05% Tween 20) for one hour at room temperature. Finally, the plate was incubated with horseradish peroxidase (HRP)-conjugated anti-human IgG- or IgM-specific secondary antibody for one hour at room temperature. The reaction was developed with o-phenylenediamine and measured using an iEMS MF microphotometer at 492 nm. Cut-off values were calculated from the average of measured optical density 492 nm data. All measurements were standardized using a monoclonal anti-citrate synthase antibody (clone 4H3-E5) produced previously.

**Serological tests for anti-bacterial antibodies**

Commercial ELISA kits were used to determine the anti-bacterial antibody concentrations in the plasma and PF samples. B. burgdorferi-specific IgG and IgM, anti-H. pylori IgA and IgG, anti-Y. enterocolitica IgA, IgM and IgG antibodies anti-C. pneumoniae and anti-M. pneumonia IgA, IgG and IgM antibodies were all measured by indirect ELISA tests according to the manufacturer's instructions. Briefly, plasma samples at 1:100 dilution and PF samples at 1 20 dilution were incubated for one hour at 37.8 $^{o}$C. The plate was then incubated with HRP- conjugated anti-human IgA/IgG/IgM antibodies for 30 minutes at 378C. The reaction was developed with 3,3',5,5'-tetrame- thylbenzidine and measured using ELISA reader at 450 nm.

**Statistical analysis**

Values are presented as mean ± SEM. Multiple groups were compared using either the Kruskall–Wallis test followed by Spearman correlation or regression analysis, as appropriate. PF and blood data were analyzed using Wilcoxon test. A value of $P < 0.05$ was considered statistically significant when comparing CABG and AVR groups. For statistical evaluation of natural autoantibodies and bacterial antibodies

among three groups, Spearman's correlation analysis and Mann–Whitney U-tests were used as appropriate. P-values < 0,05 were considered significant.

## RESULTS

1. **Cellular composition**: Lymphocytes represented the dominant cell type in PF (61.94%). The granulocyte cell ratio was significantly lower, while both monocyte and eosinophil cell ratios were higher in PF than in blood. All the investigated lymphocyte subpopulations could be detected in the PF in different ratios than in blood. Based on the Spearman correlation, the levels of the following lymphocyte populations correlated in PF ($r \geq 0.4$; $p \leq 0.009$) with the same type of cell in blood: Tc, Treg, Tnaive, Tmemo, γ/δ T.
2. **Basic lymphocyte populations:** The level of CD8+$T_C$ cells and NK lymphocytes were significantly elevated in PF compared to blood, whereas the levels of $CD4^+T_H$ cells were significantly lower in PF than in blood. In analyzing the B cell subsets, we found lower frequencies of both B1 and B2 B cells in PF compared to the blood ($P < 0.001$ and $P < 0.002$, respectively), but the proportion of B1 cells in the $CD19^+$ B cell population was significantly higher in the PF than in the blood of cardiac surgery patients, which might be the source of natural anti-CS IgM autoantibody production in the PF.
3. **Activated and naive/memory lymphocyte subpopulations:** With a level of at least 50%, $CD45RO^+$ Tmemo cells represented the dominant lymphocyte group in PF and blood. Blood contained at least twice as many $CD45RA^+$ T naive cells as PF. If we compare the activated T cell subpopulations, $CD3^+HLADR^+$, and $CD8^+HLADR^+$ $T_C$, the cell ratio is higher in PF than in blood, while the $CD4^+CD25^+$ activated $T_H$-cell ratio is lower in PF than in blood. $CD4^+CD25^+$ activated $T_H$ ratios were significantly higher in CAD group both specimens, compared to the AVR group.
4. **Minor (regulatory) lymphocytes**: In both groups, PF contained at least 3.1 times more iNKT cells than blood. A significantly higher Treg ratio could be observed in both PF and blood of CABG patients compared to PF and blood of AVR subjects.

5. **IgA, IgG, and IgM subclasses:** We determined that IgA, IgG1-4, and IgM immunoglobulins were present in the PF. The levels of all the measured immunoglobulin classes were at least 3.5 times higher in the plasma than in the PF, but the plasma/PF ratios were different.
6. **Inflammatory cytokines:** IL6 and MCP1 had a significantly higher concentration in PF compared to plasma, while IL8 levels showed the contrary. The plasma level of MCP1 in CABG patients was significantly elevated compared to AVR group.
7. **Procoagulation markers:** SCD40L, tPA, and sP-sel were present in the PF and reached significantly higher concentrations in plasma. The sCD40L concentration in plasma was on average 133.93 times higher than in PF. In both specimens sP-sel was the dominant marker When correlating the cardiovascular factor concentrations within PF and plasma, only sP-sel showed positive correlation ($r=0.38$; $p<0.05$). No significant difference could be found between the patient groups
8. **Anti-CS natural autoantibodies:** The anti-CS IgG level was approximately 11-fold higher in plasma than in PF ($P < 0.001$), whereas anti-CS IgM concentration was only approximately two-fold lower in the PF than in the plasma ($P < 0.001$). This reflects a relative excess of anti-CS IgM autoantibodies in the PF of cardiac surgery patients. Similar differences in the levels of anti-CS IgG and IgM autoantibodies were detected in the different disease groups. A statistically significant correlation of anti-CS IgM antibody concentrations ($P < 0.001$) and a strong correlation in the case of anti-CS IgG levels ($P < 0.001$) were found between the plasma and PF ($r^2 = 0.880$ and $r^2 = 0.997$, respectively).

9. **Anti-bacterial antibody titers:** All patients were positive for at least one anti-bacterial antibody (IgG and/or IgA) in the plasma, while in the PF we found fewer positive samples, even though their corresponding plasma sample was positive. The highest proportion of positivity for anti-bacterial antibodies in the plasma was against *M. pneumoniae* (74.2%) and *H. pylori* (74.2%), whereas in the PF it was against *H. pylori* (37.0%), *M. pneumoniae* (14.8%) and *Chlamydia pneumoniae* (14.8%). No IgM positivity could be detected either in plasma or PF, which indicates the absence of an early infection. We found significantly elevated anti-

CS IgM levels in the plasma (P = 0.031) and an increased tendency in the PF (P = 0.076) of patients with positive *M. pneumoniae* antibodies. In CABG patients without previous MI there was a significant correlation between anti-CS IgG levels and *M. pneumoniae* antibody titers, while in CABG patients with previous MI *C. pneumoniae* and *B. burgdorferi* antibodies correlated significantly with anti-CS IgG levels. In patients with AVR, anti-CS IgG did not show a correlation with any of these anti-bacterial antibody levels.

# DISCUSSION

The significantly elevated number of $CD25^+$ activated $CD4^+T_H$ cells in the PF of CABG patients compared to AVR patients is in line with the earlier observation in mice experiments that $CD4^+$ T-lymphocytes may be activated by cardiac ischemia, presumably driven by recognition of cardiac autoantigens, and facilitate wound healing of the myocardium.

Earlier investigations proved the ability of Treg cells to suppress proatherogenic effector T cells in atherosclerosis. Studies reported reduced circulating Treg cell numbers in patients with acute coronary syndrome compared to patients with stable angina (SA). Our results also suggest that chronic myocardial ischemia with SA (our CABG group) may lead to elevated Treg cell level, not only in the blood but also in the PF because the Treg population in CAD patients compared to AVR group was significantly higher in both specimens.

INKT subpopulation is activated by both self and exogenous lipid antigens and are considered to be proatherogenic. INKT subset reached a significantly higher concentration in PF compared to blood in each case. This data supports that the pericardial space has its own, independent iNKT pool. Moreover, based on statistically significant correlations, circulating iNKT cells might influence the level of other lymphocyte subgroups in both blood and PF.

Tmemo lymphocytes conventionally are a feature of an immune response against foreign antigens. However, there is evidence in mice that iNKT cells can induce mature naive $CD8^+$ T cells to acquire innate characteristics in peripheral

lymphoid tissue via IL4. This latter crosstalk could be a possible explanation for the high ratios of $T_c$ and iNKT subpopulations in the pericardial space.

Studies have shown that B1 cells are atheroprotective, mainly via the production of natural IgM antibodies that bind oxidized low-density lipoprotein and apoptotic cells. B2 cells are suggested to be proatherogenic. After AMI in mice, mature systemic B lymphocytes induce monocyte mobilization and recruitment to the heart, leading to enhanced tissue injury and deterioration of myocardial function. We found an amount of B2 lymphocytes in PF of CABG patients 1.88 times higher than in the aortic valve group. The B2 cell number in systemic blood flow showed no such difference, suggesting that the B2 lymphocyte population of the PF might have a role in CAD related myocardial ischemia.

The distribution of PF immunoglobulin levels were similar to that of blood; however, the PF contained significantly less IgA, IgG1-4, and IgM compared to plasma. Here we uncover for the first time the Ig composition of the PF of cardiac surgery patients. It was earlier described that after ischemia and reperfusion of the heart, complement is activated by self-reactive natural IgM antibodies that bind to postischemic tissue .

The significantly different distribution of markers of immune activation in PF and plasma implies the presence of local factors that influence the concentration of cytokines in the PF. All of the investigated cytokines have a molecular weight under 40kDa, making diffusion through membranes possible, except for tPa (68 kDa). The half-life of MCP1, tPA, sCD40L, and IL8 are shorter (maximum 20 minutes) in the bloodstream, whereas sP-sel (100 minutes) and IL6 (maximum 6 hours) last longer. This could explain why sP-sel is the only factor with correlating concentrations between plasma and PF. Our data confirmed that the PF reflects the composition of cardiac interstitium.

The strong positive correlation between pericardial MCP1 and IL6 levels and the fact that, exclusively, these biomarkers had higher concentrations in PF when compared to blood demonstrates the local significance of these cytokines. Our data of elevated MCP1 in CABG patients is congruent with the results of Yu et al., who described that shear stress (endothelial activation, first step to atherosclerosis)

increases the secretion of MCP1 in human umbilical vein endothelial cells. Our results also revealed that circulating MCP1 also seems to act as an important link between PF and plasma lymphocytes.

Even though plasma inflammatory biomarkers may not adequately reflect local tissue levels, earlier studies measured the concentrations of peripheral venous or coronary blood and suggest that compounds with key roles in plaque-destabilization and MI are also released from non-coronary sources; therefore, further PF analysis is needed.

. The increased level of anti-CS IgM may be due to the presence of increased frequencies of B1 cells in the PF. B1 cells are the probable source of natural IgM.

Our data suggests that the PF within the pericardial space is protected by the natural immune system, which may play a crucial role in tissue homeostasis by the elimination of cellular and molecular debris ( including CS) that needs to be cleared in order to prevent the accumulation of apoptotic material to control inflammation and autoimmune mechanisms, and to prevent their 'toxicity' to cardiomyocytes. Clinical data obtained from patients with systemic lupus erythematosus also support the notion that natural IgM autoantibodies may inhibit carotid atherogenesis.

The strong correlation of anti-CS IgG in plasma versus PF may suggest that these antibodies may not be produced exclusively in the PF, but they could be transported from the plasma or lymphatic fluid into the pericardial space. On the other hand, our data showing the relative increase of anti-CS IgM antibody in the PF is consistent with the notion that B1 cells produce anti-CS IgM (but not anti-CS IgG) locally in the pericardial space.

All patients were positive for antibodies to at least one bacterial species (*M. pneumoniae-, Y. enterocolitica-, C. pneumoniae-, H. pylori-,* and *B. burgdorferi*) however, some patients were negative for some of these antibodies in the PF. This may suggest that the levels of anti-bacterial antibodies in the plasma may be generally higher than in the PF (the PF is more sequestered from the immune system than blood), resulting in antibody levels below the limit of detection in the PF. Interestingly, *M. pneumoniae* has been found in atherosclerotic plaques and in normal

arteries and veins of patients, and this bacterium was also shown to be present in the PF of atherosclerotic patients.

*H. pylori, C. pneumoniae* have also been found in human atherosclerotic plaques. Our current data on the high frequency of antimicrobial antibodies in the plasma and PF of patients undergoing heart surgery (atherosclerosis-related coronary heart disease) is consistent with the potential pathogenic role of the above-mentioned bacteria in these diseases.

Our data suggests an interrelationship between natural and anti-bacterial antibodies. We found that all patients whose plasma and/or PF were positive for antibodies to *M. pneumoniae* had significantly higher levels of anti-CS IgM, both in their plasma and PF. Investigating the different disease groups, in CABG patients without previous MI we found a significant correlation between anti-CS IgG levels and *M. pneumoniae* antibody titers, while in CABG patients with previous MI, *C. pneumoniae* and *B. burgdorferi* antibody concentrations showed correlation with anti-CS IgG levels..

The correlation of elevated anti-CS IgM levels with the *M. pneumoniae* infection was independent from the disease groups. This may reflect its general protective role as a natural autoantibody, independent of the presence or absence of atherosclerosis. In contrast, anti-CS IgG level showed a strong correlation with higher levels of atherosclerosis-related anti-bacterial antibodies only in patients with coronary artery disease, which reflects its potential protective role in these infections. This data suggests that the PF is being 'surveyed' by the humoral components of the natural and adaptive immune systems in strong cooperation to protect the pericardial space from accumulation of toxic molecules and various pathogenic microbes.

# Publications related to this dissertation

**Gilicze, O**., Simon, D., Farkas, N., Lantos, M., Jancso, G., Berki, T., & Lenard, L. (2019). Characterization of lymphocyte subpopulations and cardiovascular markers in pericardial fluid of cardiac surgery patients. *Clinical Hemorheology and Microcirculation*, 1–12. https://doi.org/10.3233/ch-190594

Simon D, **Gilicze O**, Farkas N, Najbauer J, Németh P, Lénárd L, Berki T (2018) 'Correlation of natural autoantibodies and cardiovascular disease-related anti-bacterial antibodies in pericardial fluid of cardiac surgery patients', *Clinical & Experimental Immunology*. John Wiley & Sons, Ltd (10.1111), 193(1), pp. 55–63. doi: 10.1111/cei.13127.

## Abstracts in peer-reviewed journals

**Gilicze O.,** Lenard L., Kvell V., Farkas N., Szabados S., Czirjak L. S (2012) 'Autoantibody and cardiovascular marker quantification in pericardial fluid and blood samples - Plenary Lectures and Subspecialty Poster Sessions', *European Journal of Clinical Investigation*. John Wiley & Sons, Ltd (10.1111), 42, pp. 1–11. doi: 10.1111/j.1365-2362.2012.02649.x.

**Gilicze O**, Simon D, Berki T, Czirják L, Szabados S, Lénárd L (2012) 'PP-093 Identification of lymphocyte populations and cardiovascular factors in pericardial fluid and blood samples among patients undergoing open heart surgery', *International Journal of Cardiology*. Elsevier, 155, pp. S129–S130. doi: 10.1016/S0167-5273(12)70313-3.

**Gilicze O**, Lénárd L, Simon D, Berki T, Czirják L, Szabados S: Kardiovaszkuláris markerek eltérő koncentrációja infarktuson átesett betegek perikardiális folyadékában. *Cardiologia Hungarica* 2012, 42: K14

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