# C-REACTIVE PROTEIN ANALYSES OF PERICARDIAL FLUID

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***This study was designed to examine the relationship between the pericardial fluid and plasma CRP levels, and the alterations in other biochemical parameters in patients undergoing coronary artery bypass grafting (CABG).***
***The study group consisted of 96 patients with coronary artery disease (CAD) who were referred to our clinic for a CABG procedure and from whom sufficient amounts of pericardial fluid could be collected. The patients were classified into 3 groups: Group 1: SAP (n=27), Group 2: USAP (n=36), Group 3: PMI (n=33). CRP, glucose, albumin, total protein, CK, CK-MB and LDH levels were determined in the pericardial fluid samples and in simultaneously collected blood samples from the radial artery.***
***The pericardial CRP levels and the serum LDH levels in the PMI group were higher compared to the SAP ($p = 0.015$ and $p = 0.000$, respectively) and the USAP ($p=0.011$, $p=0.047$) groups. The pericardial albumin levels in PMI group was higher compared to the USAP group ($p=0.038$). Serum CRP levels in the USAP ($p=0.014$) and PMI ($p=0.000$) groups were higher than those in the SAP group. In all groups, pericardial fluid/serum protein ratio was > 0.5, LDH ratio was > 0.6, and the pericardial fluid LDH concentrations were > 300 mg/dl. These results indicate that the pericardial fluid has the characteristics of an exudate.***
***Following myocardial infarction, the concentration of CRP, which is a molecule that has no local production and is larger than 40 kD, rises in pericardial fluid due to the increased permeability of epicardium. In the pericardial fluid, the concentrations of enzymes like LDH or CK-MB that have a large molecular size and that are released upon the injury of cardiac myocytes exceed those in serum.***

***Key words: Pericardial fluid, CRP, pericardial CK-MB, pericardial LDH, exudate***

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Pericardial fluid, which is located in the space between the parietal and visceral layers of the pericardium and produced by the visceral pericardium has a volume of approximately 15-50 ml and has the function of lubrication [1-3]. Since the content of this fluid is similar to that of plasma, it is considered as an ultrafiltrate of plasma and the concentrations of many molecules are close to those in plasma [2-4]. Many factors including systemic diseases, coronary artery disease, malignant diseases, connective tissue disorders, infections, and idiopathic causes can increase the amount and change the composition of pericardial fluid [1,3-5,7,8]. The main reason behind the scarcity of studies analyzing the pericardial fluid is the difficulty of collecting

pericardial fluid samples. The importance of diagnostic studies in pericardial fluid was emphasized by Myers et al. [5], and Burgess et al [6] which drew our attention to the usefulness of the biochemical parameters in the differential diagnosis of pericardial effusions.

Recent studies provide evidence that inflammation plays a role in the pathogenesis of cardiovascular disease. Baseline levels of C-reactive protein (CRP) in apparently healthy peeple or patients with stable angina pectoris constitute an independent risk factor for cardiovascular events, whereas the rise in CRP after acute myocardial infarction (AMI) or during unstable angina pectoris (USAP) correlates with outcome. The link between CRP and cardiovascular disease is thought to be indirect in that circulating CRP only reflects the extent of acute phase reaction in response to non-specific stimuli such as confound risk factors, atherosclerosis, vascular injury, ischemia and necrosis [9-12].

This study was designed to examine the relationship between the concentrations of CRP in serum and pericardial fluid, and other biochemical parameters in patients undergoing coronary artery bypass grafting (CABG).

## MATERIALS AND METHODS

Out of 145 consecutive CAD patients referred to our clinic for a CABG procedure, 96 patients with a volume of pericardial fluid sample exceeding 10 ml were included in the study. Patients with a sample volume less than 10 ml, and patients on insulin or oral anti-diabetics were excluded (n=49). Informed consent was obtained from the patients and Institutional Ethics Committee approved the study protocol.

Patients were classified into 3 groups:

Group 1: Patients with stable angina pectoris (SAP) who were operated on the basis of critical narrowing detected by coronary angiography (n=27);

Group 2: Patients with a diagnosis of unstable angina pectoris (USAP) (n=36); and

Group 3: Patients suffering from recent (< 4w) MI (post-myocardial infarct=PMI) (n=33)

CRP, glucose, albumin, total protein, CK, CK-MB and LDH levels were determined in the pericardial fluid samples and in simultaneously collected blood samples from the radial artery. In order to estimate the percentage of CK-MB in total CK, percent relative index (PRI) was used.

$$PRI = \frac{CK\text{-}MB}{CKtotal} \times 100$$

CRP levels were determined by turbidimetric methods; glucose and protein were measured by ion-selective methods; and albumin, LDH, CK and CK-MB were measured by spectrophotometric methods.

## STATISTICAL ANALYSES

The results were expressed as mean ± standard deviation. All analyses were performed using the SPSS software for windows (SPSS Inc, Chigaco, IL, USA) and the differences were considered statistically significant at a probability level of less than 0.05.

Results in the three groups were compared with repeated measured analysis of variance (ANOVA) followed by Bonferroni post-hoc test.

## FINDINGS

There were no significant differences between groups with regard to age, gender and BMI (Table 1). Ejection fraction (EF) was significantly lower in PMI group compared to USAP group (p=0.007).

Results of biochemical analyses in pericardial fluid and arterial samples are shown in Table 2.

The pericardial fluid CRP and serum LDH levels were significantly higher in PMI group

**Table 1.** Characteristics of patients

| | SAP (n = 27) | USAP (n = 36) | PMI (n = 33) |
|---|---|---|---|
| Age (Year) | 58.8±7.2 | 60.2±9.6 | 55.4±9.6 |
| Gender (M/F) | 19 / 8 | 25 / 11 | 24 / 9 |
| BMI | 27.7±3.4 | 28.3±3.6 | 26.8±2.8 |
| EF (%) | 55.5±14.1 | 56.9±14.2 | 45.7±10.1* |

*vs. USAP, p=0.007 (One way ANOVA test)

**Table 2.** Results of pericardial fluid and simultaneous blood samples

| | SAP | USAP | PMI |
|---|---|---|---|
| **PF** | | | |
| CRP(mg/L) | 3.1±1.1 | 3.5±1.6 | 7.7±8.5a,b |
| Glucose(mg/dl) | 110.8±32.7 | 113.8±16.2 | 105.0±24.9 |
| LDH(mg/dl) | 374.6±84.4 | 666.4±60.5 | 814.1±72.2c,d |
| CK(U/L) | 56.4±6.3 | 49.9±9.5 | 73.8±23.3 |
| CK-MB(U/L) | 11.8±5.9 | 19.9±4.6 | 25.0±3.2 |
| Alb(mg/dl) | 2.5±0.6 | 2.3±0.6 | 2.8±0.8e |
| Prot (mg/dl) | 3.9±1.1 | 6.8±1.1 | 4.2±1.3 |
| **Blood** | | | |
| CRP(mg/L) | 4.7±1.8 f, g | 8.3±1.7 | 13.0±1.9 |
| Glucose(mg/dl) | 130.8±10.6 | 155.6±17.6 | 135.1±11.9 |
| LDH (mg/dl) | 360.7±13.6 | 321.2±26.8 | 353.1±17.7 |
| CK (U/L) | 108.7±8.6 | 118.6±12.3 | 137.3±20.1 |
| CK-MB (U/L) | 8.7±4.3 | 9.6±3.1 | 9.9±4.1 |
| Alb(mg/dl) | 4.2±0.3 | 4.0±0.6 | 4.1±0.7 |
| Prot(mg/dl) | 7.0±0.7 | 8.6±1.0 | 6.7±1.0 |

compared to the SAP group (p=0.015, p=0.000) and the USAP (p=0.011, p=0.047) groups. In the PMI group, the albumin concentration in the pericardial fluid was higher than in the USAP group (p=0.038). The serum CRP levels in the SAP group were significantly higher compared to those in the USAP (p=0.014) and in PMI (p=0.000) groups.

CK-MB PRI levels in the pericardial fluid (SAP=21, USAP=40 and PMI=34) were higher than in the serum (SAP= 8, USAP = 8 and PMI= 7). While the ratios in the serum were similar across the study arms, the pericardial fluid levels were remarkably high in the USAP and the PMI groups.

The serum protein and LDH ratios for the pericardial fluid are depicted in Figure 1. In all groups, the pericardial fluid/serum protein ratios were larger than 0.5, and the pericardial fluid/serum LDH ratios were larger than 0.6. Furthermore, the LDH concentrations in the pericardial fluid were above 300 mg/dl in all groups (Table 2). These results demonstrate that the pericardial fluid in all three arms of the study has the characteristics of an exudate based on Light's criteria.

## DISCUSSION

In this study, serum CRP levels in USAP and PMI groups were higher compared to levels in SAP group, but in only PMI group the pericardial fluid concentrations were above the levels in other groups. In line with serum CRP levels, the concentrations of LDH in pericardial fluid were higher in the USAP and PMI groups.

The C-reactive protein has many biologic activities related to non-specific host defenses. It acts as an opsonin for bacteria, parasites and immune complex and can activate the classic pathway of complement. Increased CRP levels reflect cytokine-mediated hepatic response triggered by an inflammatory stimulus [10].

In patients with myocardial infarction, serum CRP levels rise and this increase can persist for more than 4 weeks [10]. In the USAP patients, CRP levels rise due to the presence of active atherosclerotic lesions [9-12]. Therefore, in patients with coronary heart disease, increased CRP levels might reflect inflammation of the arteries that is associated with changes in plaque morphology, rupture and thrombosis [10].

In this study, ongoing inflammatory processes in both groups has a major role in higher serum levels of CRP observed in the USAP and PMI groups compared to SAP group. High concentrations of CRP in pericardial fluid was detected only in PMI group and patients in the USAP group had normal levels; these findings underscore the importance of epicardial diffusion, in which only molecules smaller than 40 kilo Daltons (kD) can readily diffuse

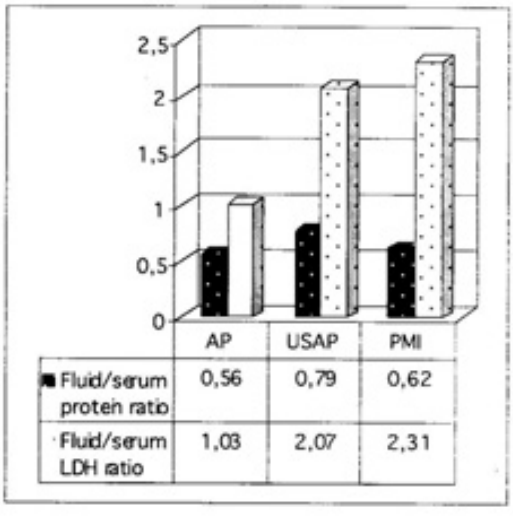


**Figure 1.** Results of pericardial fluid analysis according to Light's criteria.

into pericardial fluid and larger molecules can not [2,4].

Human CRP cannot enter the pericardial cavity by epicardial diffusion owing to its pentameric structure with a molecular weight of 118 kD. However in conditions like AMI or agonal myocarditis, it can diffuse into the pericardial cavity due to local vasodilation and increase in permeability [13].

Interleukin (IL)-1, IL-6 and TNF_ released upon activation of defense mechanisms of the body can stimulate the production of CRP in the liver. Within 24 hours of the stimulation, the circulating levels of CRP increase considerably [14-16]. Since there is no local production of CRP in the pericardial cavity, it is possible that molecules in this fluid may have diffused by altered epicardial diffusion.

CRP levels in USAP patients were high in serum, but low in pericardial fluid; this finding suggests that epicardial diffusion is preserved in this group of patients.

The distinction between exudate and transudate is usually based on Light's criteria. A fluid/serum protein ratio greater than 0.5, a fluid/serum lactate dehydrogenase (LDH) ratio greater than 0.6 and a fluid LDH concentration greater than 300 U/L is indicative of an exudate. If the total protein content in the fluid is greater than 3.0 g/dL the sensitivity is 97%, and if the fluid/serum protein ratio is greater than 0.5 the sensitivity is 96%. A LDH level greater than 300 mg/dl is 98% sensitive for an exudate; and fluid/serum LDH greater than 0.6 is 94% sensitive for an exudate [3,5,17].

In our study, in all 3 groups of patients the pericardial fluid samples had the characteristics of an exudate based on Light's criteria. The high LDH levels and fluid/serum LDH ratios in USAP and PMI groups were remarkable.

LDH is an oxidoreductase enzyme with a molecular weight of 140 kD that converts lactate to pyruvate, and is classified into 5 subgroups based on the differences in its subunits. While under normal conditions LDH1/LDH2 ratio is greater than 0.7, in angina and cardiac dysfunction the LDH level rises and this ratio increases up to 0.8-1.0 accordingly. This ratio is an important parameter used for transudate-exudate distinction. LDH levels above 200 U/L are considered to show the presence of an exudate when Light's criteria are applied for transudate-exudate distinction [1,3,5-8,17]. On the other hand Strimel et al. [3], Meyers et al. [5], and Atar et al. [7] adopt a LDH level of greater than 300 U/L for the characterization of exudates.

Because of the probability of an increased diffusion of LDH into the pericardial fluid due to an abnormal epicardial permeability that increases the total LDH level, we believe that, pericardial fluid should be evaluated in a different way than other body fluids while examining the LDH level in fluid, which is considered to be the most sensitive marker for transudate-exudate distinction based on the Light's criteria. A study of subgroups of LDH enzyme in pericardial fluid would shed more light on this subject.

Meyers et al. [5] report that glucose levels are higher when the pericardial fluid has the characteristics of an exudate, and that pericardial fluid/serum glucose ratio is $> 1$ when the fluid is a transudate and $< 1$ when it is an exudate. In our study, the glucose levels in pericardial fluids in all groups were lower compared to serum levels.

Spodick [1] reports that the protein content of pericardial fluid is lower compared to that of plasma, and that albumin levels in pericardial fluid may be higher compared to plasma owing to its lower molecular weight and its ease of transport. In our study, serum levels of albumin and protein were higher compared to those of pericardial fluid in all groups. This finding is in line with the previously reported results.

Creatinine kinase (CK) is composed of 2 subunits, each with a molecular weight of 43 kD. Different localization of these two subunits gives rise to three distinct isoenzymes: CK-MM, CK-MB, and CK-BB are the predominant isoenzymes in skeletal muscle, cardiac muscle, and brain, respectively. CK-MM is responsible from more than 95% of total CK, and CK-MB is responsible from less than 5%; the proportion of CK-BB in total CK level is near 0%. Muscle trauma, myositis, and cardioversion increase the circulating levels of CK [18-21].

In our study, the concentrations of CK in pericardial fluid were lower compared to serum levels, whereas the CK-MB levels in pericardial fluid were higher. CK-MB PRI in the serum was similar across the three groups, but the CK-MB PRI in pericardial fluid was significantly higher in USAP and PMI groups. The high level of CK-MB PRI is suggestive of an excess of CK-MB in total CK levels. Also, the high ratio detected in USAP and PMI groups demonstrate the increased release of this enzyme from the myocytes.

In conclusion, the USAP and PMI groups had increased levels of serum CRP due to the inflammatory processes, whereas only the PMI group had an increased level in pericardial fluid due to the abnormal epicardial diffusion. Large molecules like LDH and CK-MB that are released upon myocyte injury have higher levels in pericardial fluid samples of USAP and PMI patients. Although these findings do not provide a new therapeutic approach, our study presents a detailed review of changes in the composition of pericardial fluid in coronary artery disease patients.

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