

SUMMARY

Introduction

Cardiovascular diseases, especially ischemic heart disease and vascular brain diseases substantially impair quality of life of affected people and are the most frequent cause of both morbidity and mortality in European countries.

The longterm chronic inflammatory reaction has been reported to be implicated in the pathogenesis of atherosclerosis. The presence of inflammatory markers detected in peripheral blood are recognized as risk factors of a development of ischemic heart disease. There are overwhelming evidences based both on the presence of inflammatory cells in the damaged arterial cell wall, and systemic presentation of this inflammatory reaction which precede the onset of acute coronary syndromes. Furthermore, there is an association of some chronic infections caused e.g. Chlamydia species with more frequent incidence of coronary arteries atherosclerosis in patients with genetically determined proinflammatory phenotypes.

The clinical presentation of ischemic heart disease is positively modulated by both minimalisation of adverse effects of risk factors during life and by the administration of various drugs. To recanalise coronary artery occluded by thrombus during acute coronary event either thrombolytic therapy or invasive percutaneous cardiologial interventions are highly effective. Severe forms of ischemic heart disease are treated by cardiac surgery using aortocoronary bypass to overpass stenosis of a coronary artery. Classical approach to such cardiac surgeries is exploiting cardiopulmonary bypass. Novel approach which is based on so called beating-heart surgery is avoiding proinflammatory effect of cardiopulmonary bypass.

There is a substantial complex disturbance of body integrity and homeostasis during cardiac surgical operation and in the early postoperative period ultimating in the development of severe and in some cases overwhelming inflammatory response. Numerous so called "danger patterns" of both endogenous and exogenous origin are raised during cardiac surgery. Identification of the danger pattern by the innate immunity via pattern recognition receptors are stimulating intracellular activation pathway, especially NF κ B transcription factor. The enhanced transcription of many genes coding for factors regulating inflammatory response is in the end of this highly sophisticated cascade.

The spectrum of danger patterns, qualitative and quantitative parameters, and dynamics of inflammation are different in patients operated either using cardiopulmonary bypass or surged on the beating heart. Many doubts remain which surgical approach is superior concerning the outcome of cardiac surgery. It is generally claimed that "beating-heart" surgery is activating an inflammatory response in a lesser magnitude compared to surgery using cardiopulmonary bypass. To reconcile this new data describing inflammatory response during cardiac surgical operations are exagerately warranted. Amongst other, it is essential to identify parameters of immune system which are displaying not only proinflammatory activities but also regulatory (homeostatic) and termination parameters of inflammatory reaction raised by cardiac surgical operation.

This thesis is summarising the results of the complex study focusing on the intensity and dynamics of selected parameters of the immune system in the representative group of cardiac surgical patients operated either with or without the use of cardiopulmonary bypass.

Patients and Methods

Forty patients referred to first-time coronary artery bypass grafting (CABG) were enrolled in this study. Patients underwent either conventional myocardial revascularization with cardiopulmonary bypass and cardioplegic arrest of the heart ("on-pump", n=20, 16 male, 4 females, mean age 69 \pm 7) or beating heart surgery ("off-pump", n=20, 15 males, 5 females, mean age 66 \pm 10). Patients in both groups were comparable in age, preoperative left ventricular ejection

fraction (median 0.65 in "on-pump", 0.65 in "off-pump" patients, respectively) and the number of performed coronary anastomoses (median 2.0 in "on-pump", 2.0 in "off-pump", respectively). Both venous blood (peripheral venous blood from an antebrachial vein) and arterial blood was withdrawn. In "on-pump" patients, blood was withdrawn at (1) introduction to anaesthesia (in both groups represented the baseline or reference value for all parameters measured thereafter), (1a) before cross-clamping of the aorta, (1b) after aortic cross-clamp release (1c) after termination of CPB, (2) after termination of the operation, (3) the first postoperative day, (4) the third postoperative day and (5) the seventh postoperative day. Samples 1a, 1b and 1c were not available in „off-pump“ patient for obvious reasons.

Serological parameters were quantitatively detected by commercially available ELISA kits. Double immunofluorescence standard whole blood staining method was used to determine surface expression of selected markers. Measurements were performed using FACSCalibur flow cytometer and data acquired by CellQuest software (BD Bioscience, NY, USA).

Statistical analysis was performed with Statistica 5.5 software (StatSoft, USA). Probability values of < 0.05 were considered statistically significant.

Aim of the study

The aim of this thesis was to assess the changes of selected immunological parameters in cardiac surgical patients who underwent coronary artery bypass grafting (CABG) either using or avoiding cardiopulmonary bypass (CPB).

The principal aims of this study were:

1. to exploit cardiac surgical operations as a clinical model of an inflammatory response
2. to add new original data to reconcile which surgical approach, "on-pump" versus "off-pump" is superior regarding development of inflammation in cardiac surgical patients

Results

In spite of nowadays generally accepted hypothesis that "on-pump" surgery is associated with more profound inflammatory response we did not find any statistically significant differences between "on-pump" and "off-pump" cardiac patients in majority of cell-mediated parameters tested. We only found significantly higher expression of scavenger CD163 receptor and activation molecule CD38 on monocytes in "on-pump" patients compared to "off-pump" patients on the 1st postoperative day. There were no significant differences in the expression of pattern recognition receptors TLR-2 and TLR-4, CD64 high affinity receptor for IgG (FcγRI), and activated form of integrin β₂ chain CD18 recognized MEM-148 monoclonal antibody between "on-pump" and "off-pump" patients.

In the case of humoral immune parameters the significant differences between "on-pump" and "off-pump" surgery were much more frequent. Such differences were found for the levels of IL-6, IL-10 interleukins, long pentraxin PTX3 and Hsp70 stress proteins, respectively. We found significantly higher concentration of these parameters at the finishing of surgery. As original finding it could be recognized the fact that the IL-6 concentration is significantly higher in "on-pump" patients, whereas there is a total switch in IL-6 level at the 1st postoperative day being significantly higher in "off-pump" patients.

Conclusion

Our results documenting the differences between "on-pump" and "off-pump" patients have not been equivocally interpreted. It could be summarized with some limitations the dynamics of an antiinflammatory (homeostatic) IL-10 is copying the dynamics of proinflammatory IL-6 cytokine or is preceding IL-6 production. It could be implicated from our results that the role of IL-13 seems to be neglected in cardiac surgery. Based on the lack of correlation between the levels

of sCD14 and LBP and IL-6, C-reactive protein and long pentraxin PTX3 concentration the former parameters are not produced as acute phase proteins in cardiac surgery. It could be delineated from our results that it is necessary to follow the dynamics of humoral parameters rather than single point measurement.

We found significant changes of both humoral and cellular parameters of the immune system which are different regarding timing and different surgical approaches. It could be implicated from our findings that different cardiac surgical strategies are responsible for different inflammatory responses during surgery and in the postoperative period.

Detailed knowledge of changes of both qualitative and quantitative parameters of the inflammatory response induced by cardiac surgical operation and their careful correlations with perioperative and postoperative clinical parameters could ultimate into the identification of the specific markers with a strong predictive value to identify the development of the deregulated harmful inflammatory response associated with the poor outcome of patients.

These markers in combination with the knowledge of genetically determined factor influencing the characteristics of the inflammatory response (e.g. TLR polymorphism) could be useful in the selection of the best individual cardiac surgery approach with the minimum of adverse effects for the patients.