Immunological and serological discrimination of children with and without post-surgical capillary leak syndrome

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SUMMARY

Children undergoing open heart surgery with cardiopulmonary bypass can suffer from post operative „capillary-leak-syndrome“ (CLS). The prognostic value of the immunstatus for CLS was analyzed. Serum cytokine concentration and immunophenotype of 9 children with and 9 without CLS were determined before, during and after surgery. Children with CLS had preoperatively and thereafter elevated levels of complement components, soluble adhesion molecules, neopterin, histamine, IL-10 and decreased C1-inhibitor concentration. Immunophenotyping showed an elevated CD4/CD8 T-cell ratio in CLS patients, elevated activation of monocytes and an increased fraction of early activated B-lymphocytes. These data indicate slight preoperative inflammatory conditions in CLS patients. The parameter patterns seem sufficiently informative for the preoperative identification for risk patients.
INTRODUCTION

The use of cardiopulmonary bypass (CPB) during open heart surgery can induce post-operative symptoms that are similar to bacterial sepsis [1]. These symptoms include CLS and in few cases multi organ failure (MOF). The reason for this morbidity is yet unknown. Most Children who develop CLS or MOF are preoperatively often clinically inconspicuous. Some authors reported, however, that children who develop CLS have a slight increase in complement activation [1], C reactive protein concentration and leukocyte count prior to surgery [2]. These findings indicate a minor inflammatory state of the CLS patients before surgery. Torre-Amione et al. [3] have shown recently that in adults cytokine levels are increased with increased „severity“ of the heart disease. We analyzed additional inflammation markers and the immunophenotype in CLS patients in order to test if these parameters are potentially useful as prognostic markers.

PATIENTS, MATERIAL AND METHODS

18 children undergoing open heart surgery with CPB in the age range from 3 to 15 years were analyzed. Both groups of children received similar anesthesia, medication and intra- and post-operative care and did not significantly differ in age, body weight, sex and duration of surgery. General anesthesia and myoplegia were performed with midazolam, fentanyl and pancuronium. CPB was performed with a Stöckert roller pump (Stöckert Instrumente GmbH, Munich, Germany) and hollow-fiber oxygenator (DIDECO, Mirandola, Italy). CLS was defined as post-operative edema and blood effusion into the pericard (CLS group). Blood samples were taken at eight points: one day before surgery (-1d), after onset of anesthesia (anesthesia), 10-30 min after onset of the CPB (CPBI), before ending of the CPB during rewarming (CPBI), 4-6 h after surgery (+4h), one (+1d), two (+2d), three days after surgery (+3d) and shortly before discharge (discharged). Routine laboratory parameters were determined. The serum concentrations of components of the complement system were measured by radial immundiffusion (The Binding Site LTD, UK). Serum levels of cytokines (IL-1, IL-4, IL-6, IL-8, IL-10, IFNγ, TNFα), and soluble adhesion molecules (ICAM-1, E-selectin, PECAM) were quantified by enzyme linked immunoassay. Immunophenotyping was performed by the whole-blood lysis method by 3- or 4-color staining. Cells were measured by flow cytometer (FACSCalibur, Becton-Dickinson, USA). Data were analyzed with the CellQuest software package (Becton-Dickinson). The percentage of different cell types and their antigen expression (detected by their fluorescence intensity above background) was determined. Statistical analysis was done with Student’s t-test, data classification with the CLASSIFI program [4].

RESULTS AND CONCLUSIONS

The routine laboratory parameters showed that leukocyte, erythrocyte and thrombocyte counts were not different in children developing CLS (data not shown). The concentration of complement components (C3, C4, C5) was slightly lowered in CLS patients and decreased furthermore during surgery (not significant, data not shown). In contrast, C1-inhibitor concentration was lower in CLS patients and remained decreased during and after surgery (Figure 1A). The concentration of soluble adhesion molecules ICAM-1 (Figure 1B), E-selectin and PECAM were preoperatively increased in CLS patients by 21%, 60% and 14%, respectively (p<0,1). These differences were sustained during and after surgery. Also the serum
levels of neopterin, histamine (Figure 1C) and interleukin-10 were elevated by 121%, 333% and 128%, respectively (p<0.1). No significant pre-, intra and postoperative values were found for the other measured cytokines. Immunophenotyping revealed a 50% increase of the CD4/CD8-ratio of T-lymphocytes in CLS patients. This ratio decreased during surgery markedly in the CLS group but did not change significantly in the CLS free group (Fig. 2A). This difference between both groups was mainly due to a decreased number of peripheral cytotoxic (CD8+ ) T-cells in the CLS group (not shown). The state of activation in peripheral blood monocytes was increased in the CLS group before and during surgery as found by the increased expression of HLA-DR (major histocompatibility complex II) (Figure 2B) and CD14 (LPS receptor) by 113% and 77%, respectively (p<0.05).

In spite of some clear preoperative differences of the serum level of complement factors, cytokines and cellular immunophenotype none of the detected differences alone could be used to clearly discriminate all of the CLS patients. We therefore classified CLS and non-CLS patients by a software package (CLASSIF1) [4] using all measured laboratory and serological values. In this first attempt flow cytometric data were excluded from the analysis. It was possible to classify all children correctly. 10 out of 34 parameters were selected by the program for classification. The result of this classification is shown in Table 1.

The classification indicated that a slight increase in the inflammation status of the children (i.e. increased neutrophil count and percentage, concentrations of interleukin-10, histamine in serum and urine, ICAM-1 and E-selectin and decreased C1-inhibitor concentration) with CLS. In addition, increased age and body weight were correlated with increased risk for CLS.

Our results demonstrate that pre-operative differences in the immunostatus are po-
Figure 2: Alterations of the CD4/CD8-ratio of T-lymphocytes and monocyte phenotype during cardiac surgery with CPB in children with (open symbols) and (without closed) post-surgical CLS. The T4/T8 ratio (A) and the HLA-DR expression of peripheral blood monocytes (B, expressed as fluorescence intensity) are displayed versus time of sampling. Further details as in figure 1.

Table 1: Classification matrix for the discrimination of children with and without post-surgical CLS by preoperative concentration of serum factors and clinical parameters.

<table>
<thead>
<tr>
<th>Clinical outcome (complications)</th>
<th>Prospective CLASSIFI1 Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Patients</td>
</tr>
<tr>
<td>CLS</td>
<td>9</td>
</tr>
<tr>
<td>no complications</td>
<td>9</td>
</tr>
</tbody>
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tentially important for the prognosis of CLS development following cardiac surgery. These differences might either be due to a slight pre-operative inflammation or are of endogenous (e.g. heart disorder induced) origin. As shown recently for adults [3] some of these parameters could be also indicators for the severity of the heart disease.

REFERENCES

