Cytometry (Communications in Clinical Cytometry) 46:247–253 (2001)

Preoperative Prediction of Postoperative Edema and Effusion in Pediatric Cardiac Surgery by Altered Antigen Expression Patterns on Granulocytes and Monocytes

Attila Tárnok,[1*] József Bocsi,[2] Michal Pipek,[1] Pavel Osmancik,[1] Günter Valet,[3] Peter Schneider,[1] and Jörg Hambsch[1]

[1]Pediatric Cardiology, Heart Center Leipzig GmbH, University Hospital, Leipzig, Germany

[2]Ist Institute of Pathology, Semmelweis University of Medicine, Budapest, Hungary

[3]Max-Planck-Institute for Biochemistry, Martinsried Munich, Germany

Postoperative edema and effusion (POEE) following cardiopulmonary bypass (CPB) surgery in children retards recovery and may aggravate postpericardiotomy (PPS), capillary leak syndrome (CLS), or multiorgan failure (MOF). Compared with complication-free children, POEE affected children have different preoperative serum levels of circulating cytokines and adhesion molecules. These levels may be used preoperatively to assess POEE, but their determination is time consuming, costly, and a substantial blood volume is required. Altered serum levels of cytokines and adhesion molecules also may be reflected in altered antigen expression on circulating blood leukocytes. The predictive potential of flow cytometric (FCM) leukocyte immunophenotyping was explored as a sensitive and fast method that required small blood samples. Blood samples taken 24 h preoperatively from 49 patients (3–18 years old) were stained with monoclonal antibodies for adhesion molecules (ICAM-1, LFA-1, Mac-1) or constitutive/activation markers (CD4, CD14, CD16, CD25, CD54, CD69, HLA-DR) and measured on a microbead calibrated FCM. Neutrophils, monocytes, and eosinophils from POEE patients express higher preoperative levels of LFA-1, monocytes, HLA-DR, and other activation markers (all $P < 0.03$). Over 89% of the patients were classified correctly by using two discriminant analysis methods (sensitivity, >76%; specificity, >86%; positive prediction, >80%; negative prediction, >83%). Granulocytes and monocytes of postoperative POEE patients exhibit significant preoperative immune activation, suggesting an increased risk for patients with atopic/allergic predisposition. Surgical trauma and CPB cause additional immune activation, leading to POEE by a summative response. Most patients at risk for POEE can be identified preoperatively by using data pattern analysis on FCM-derived parameters. Cytometry (Comm. Clin. Cytometry) 46:247–253, 2001. © 2001 Wiley-Liss, Inc.

Key terms: cardiopulmonary bypass; adhesion molecules; immunology; data mining; predictive medicine

The extensive contact between blood and foreign surfaces of the extracorporal circuit during cardiopulmonary bypass (CPB) surgery, combined with medication, may lead to the stimulation of the immune system (1,2). This stimulation may cause postoperative complications such as postoperative edema and effusion (POEE), postpericardiotomy syndrome (PPS), capillary leak syndrome (CLS), or multiple organ failure (MOF; 2–5). Preoperative identification of at-risk patients can provide the rationale for individual prophylactic treatment prior or during surgery.

Several scoring systems, including clinical and laboratory parameters acquired during or after surgery, are used to predict the outcome of cardiosurgical interventions (6–8). In adults, they are based on serum parameters changes of C-reactive protein (CRP; 9), interleukin (IL)-6 (10), E-selectin (11), or lactate (12).

It is desirable to predict postoperative complications, induced by an activated immune system (1,9,13), from a

Part of this work was presented at the 71st scientific session of the American Heart Association, November 1998, Dallas, TX (Abstract 686).

Grant sponsor: Deutsche Stiftung für Herzforschung; Grant sponsor: the Deutsche Herzstiftung; Grant sponsor: Sächsisches Ministerium für Wissenschaft und Kunst.

*Correspondence to: Dr. Attila Tárnok, Pediatric Cardiology, Cardiac Center Leipzig, University Leipzig, Russenstr.19, D-04289 Leipzig, Germany.

E-mail: tarnok@medizin.uni-leipzig.de

Received 17 January 2001; Accepted 17 May 2001

predisposition by the cardiac disease (14,15) or from other causes (e.g., atopy/allergy; 16,17) already present preoperatively (7). Predisposed patients may respond more dramatically to CPB and surgical trauma. Similarly, patients at risk for postoperative blood loss already have altered blood coagulation prior to CPB (18). Using two data classification programs (20), POEE-susceptible children were identified by altered 24-h preoperative serum levels of seven circulating cytokines and adhesion molecules (19). The significant time involved, the high blood volume required, and the expensive cost prohibit routine clinical application.

The determination of leukocyte activation markers by flow cytometry (FCM) as a rapid and economical method that requires a small blood sample seems to be a promising alternative. The prognostic value of cellular parameters was shown for restenosis (21) and neonatal sepsis (22). Preoperative humoral changes in blood serum (19), in conjunction with altered leukocyte adhesion or activation molecule expression, may cause reperfusion injury and CLS (21,23,24). The purpose of the present study was to examine 24-h preoperative granulocyte and monocyte marker differences, associated with POEE development, to facilitate preoperative identification of patients at risk for POEE.

MATERIALS AND METHODS

Patients, CPB, and Patient Classification

The study was conducted between November 1995 and January 1999. Forty-nine children underwent elective cardiac surgery with CPB for the repair of congenital heart disease. The study was approved by the ethical committee of the medical faculty of the University of Leipzig. All consecutive pediatric patients who underwent cardiac surgery during the time period of the study and fulfilled the inclusion criteria (ages 3–18 years, body weight >12 kg, parental consent, surgery with CPB) were included in the study. Only 25% of the children who fulfilled the inclusion criteria age, weight, CPB surgery were included in the study because parental consent was not obtained. The surgical procedures were closure of atrial (n = 22) or ventricular septal defect (n = 7), pulmonary homograft (n = 8), Glenn or Fontan operation (n = 6), membrane resection (n = 3), correction of pulmonary stenosis (n = 2), and tetralogy of Fallot (n = 1). All children received similar anesthesia, medication, and intraoperative and postoperative care (2). For all operations, general anesthesia was performed with intubation and intravenous administration of dormicum, fentanyl, propofol, etomidate, and pancuronium over the central vein for maintenance. Anesthesia was applied by the same anesthesiologist. Although anesthesia conditions were similar for all patients, identity was not achievable due to differences in the length of the various operations. After delivery to the pediatric cardiac intensive care unit (ICU), the incidence of the following symptoms during the first 24 h up to Day 3 after surgery were monitored: pericardial, pleural, and peritoneal effusion were measured by ultrasonography; liver swelling of more than 1 cm above preoperative values, determined by subcostal palpation along the medioclavicular line, was monitored by one of us (J.H.); and edema of the face, hands, and feet, was monitored by one of us (J.H.) based on clinical appearance.

According to these symptoms the patients fell either into a control group (n = 28; no measurable edema/effusion [n= 19], edema of the face, hands, or feet [n= 5], liver swelling <1 cm [n = 4]) or into the POEE group (n = 21; pericardial, pleural, and/or peritoneal effusion alone or in conjunction with edema and/or liver swelling >1 cm [n = 17], liver swelling >1 cm with edema of the face and/or hands and feet [n = 4]). None of the patients suffered from severe complications such as CLS or MOF, nor did any develop PPS.

FCM

Blood was collected 24 h before surgery in syringes containing EDTA. Leukocyte and differential blood cell counts were determined. Leukocytes were immunophenotyped by the whole blood technique (25). Blood (40 μl) was mixed with the appropriate volume of directly fluorescence dye-conjugated monoclonal antibodies at pretitered optimal concentration and stained for 15 min at room temperature (RT) in the dark. The assays were centrifuged at 300×*g* following addition of 1 ml lysing solution (BD Biosciences, San Jose, CA), mixing, and further incubation for 10 min at RT in the dark. The supernatant was discarded, the cell pellet was washed twice by centrifugation in 1 ml phosphate-buffered saline (PBS; Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany), and resuspended in 500 μl PBS containing 0.1% (w/v) paraformaldehyde (Sigma). The antibody panel included a combination of constitutive and activation-dependent leukocyte membrane antigens. Considering granulocytes and monocytes as potentially major cellular contributors to the induction of clinical complications, activation antigens (CD14, CD16, CD25, CD45, CD69, HLA-DR) and adhesion molecules on these cells were selected (CD11a, CD11b, CD18, CD54). Antibodies were obtained from BD Biosciences, Beckmann-Coulter (Hialeah, FL), Caltag (Hamburg, Germany), or DAKO (Glostrup, Denmark) and used in the following combinations: (1) CD45/CD14/HLA-DR/CD3, (2) CD25/CD54/CD3/CD19; (3) CD11a/CD18/CD16+CD56; (4) CD11b/CD18/CD3; (5) CD3/CD69/CD19/CD45; (6) CD3/CD4/CD45RA/CD45RO; and (7) appropriate isotype control antibodies. Antibodies were labeled with the fluorescent dyes fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP™), and allophycocyanin (APC). Cells were measured immediately after staining on a dual-laser FCM (FACSCalibur, BD Biosciences), calibrated with quantitative calibration beads (Spherotech, Libertyville, IL) if necessary.

Data Analysis, Statistics

FCM data were analyzed with the CellQuest software package (BD Biosciences). The monocyte, neutrophil, and eosinophil cell populations were characterized by forward and sideward angle light scatter and autofluorescence (eosinophils; 25). The percentages of the leukocyte

Table 1
Patient and Surgical Data (M ± SD)

	Control	Complication (POEE)	*P* value*
Age (years)	8.29 ± 2.98	9.59 ± 3.43	ns
Weight (kg)	26.3 ± 10.7	32.7 ± 13.8	ns
Gender F/M (n)	14/14	9/12	ns**
Aortic cross-clamping (min)	36.1 ± 31.3	38.5 ± 27.4	ns
CPB (min)	67.2 ± 41.7	90.3 ± 48.3	ns
Surgery + anesthesia (min)	177.4 ± 62.2	241.8 ± 118.2	0.017
Reperfusion (min)	14.9 ± 14.3	21.2 ± 23.5	ns
Hypothermia (minimal temperature °C)	31.0 ± 2.9	29.0 ± 4.9	ns
Stay on ICU (days)	1.89 ± 1.10	3.42 ± 2.78	0.011
Discharge (days after surgery)	6.20 ± 1.38	8.33 ± 4.42	0.030

*Two-tailed Student's *t*-test.
**Chi-square test: ns, not significant.

populations as well as their respective fluorescence intensities as measures for antigen expression were determined (26). Absolute cell counts were calculated (26) from the percentages and the absolute leukocyte counts determined by routine laboratory analysis. The number of bound antibodies per cell was determined as the difference between the mean fluorescence intensities of the specific assays and the mean intensity of control antibody-stained assays and calculated using calibration beads as the counting standard. This resulted in 122 data columns of original (n = 67) and calculated data. In addition to the data obtained by FCM, surgical and patient data (age at surgery, body weight, duration of surgery, CPB and aortic cross clamping, hypothermia, duration of hypothermia, excreted volume of urine, priming volume of the CPB) also were used for the following calculations. Between-group comparison was done by unpaired Student's *t*-test or Mann Whitney U-test as appropriate (Statistical Program for Social Sciences [SPSS], Knowledge Dynamics, Canyon Lake, TX).

For the retrospective-prospective classification, the data were distributed into a learning set and an unknown embedded test set that was obtained a priori by removing every 5th patient of each category from the learning set. Classification was performed either manually by multivariate discriminant analysis (27; SPSS, Version 8.0) or automatically by the triple-matrix data pattern analyzer CLASSIF1 (20). For the SPSS classification, the parameter with the highest between-group discrimination (average recognition index [ARI]) was selected initially. The classifier was optimized subsequently by adding more parameters until the ARI was maximal. The unstandardized canonical discriminant function was then determined (27). CLASSIF1 classification (20) was optimized between the 10% and 30% percentiles. The robustness of both classifiers was tested on the embedded test set of 11 clinically categorized patients unknown to the classifiers. Risk assessments for unknown patients at other institutions can be calculated for test purposes with the indicated formula (SPSS, see Table 4). Each patient parameter value is multiplied by a correction factor. The factor is obtained as the ratio between the respective parameter mean from the local reference group of 20–40 complication-free patients selected according to the same inclusion criteria and as the patients used for the determination of the respective reference means (see Table 2).

RESULTS

Patient Data

Surgical data are shown in Table 1. POEE patients required a significantly longer surgery, anesthesia time, and ICU stays. Other parameters, including duration of hypothermia and hemofiltration, priming, and infusion volume (not shown), were not significantly different. In agreement with earlier findings (2) and results from others (4), patients who developed complications had an extended stay in the ICU and in the hospital. All patients were discharged in good condition between 3 and 14 days after surgery.

Immunological Data

Antigen expression on leukocytes from patients with and without POEE was different. Examples of altered antigen expression are shown for two patients who underwent surgery in the same week (Fig. 1). POEE patients exhibited increased CD18, CD11a, and CD11b expression on neutrophils, eosinophils, and monocytes, respectively. CD45RA expression on eosinophils was decreased. The significantly different values of the 122 parameters are summarized in Table 2. POEE children showed preoperatively increased activation and adhesiveness of monocytes, neutrophils, and eosinophils as evidenced by the elevated antigen expression. Differential blood counts did not differ between the groups except for increased percentage and cell count of eosinophils. None of the patients had increased (>2 mg/ml) serum concentration of CRP and white blood cell count (not shown).

Data Classification

The use of a single parameter for individual risk assessment is insufficient because most of the values (~80%) of at-risk patients were similar to those of the controls (Fig. 2). Risk assessment based on the classification of the most discriminant measured or calculated parameters by the SPSS or CLASSIF1 programs classified correctly most patients (Table 3). The ARI was 89% (sensitivity, 87%; spec-

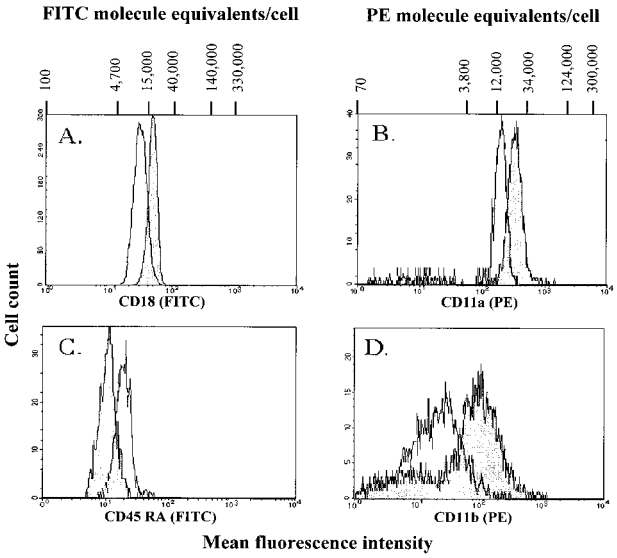

FIG. 1. Preoperative antigen expression on neutrophils **(A)**, eosinophils **(B,C)**, and monocytes **(D)** of two children, one with (closed histograms) and the other without POEE (open histograms). The patients underwent cardiovascular surgery for congenital heart disease in the same week. Data display antigen expression (fluorescence intensity, x-axis) versus cell count (y-axis) measured by flow cytometry. The top scale shows the corresponding numbers of equivalent FITC (A,C) and PE (B,D) molecules determined with standardized microbeads.

ificity, 91%; positive prediction, 91%; negative prediction, 87%) using only six of all analyzed parameters (Table 4) by SPSS. No parameters from neutrophils were selected. CLASSIF1 classified correctly 90% of the patients (sensitivity, 76%; specificity, 92%; positive prediction, 86%; negative prediction, 83%) and selected 10 parameters for masks and classification (Table 4). Monocyte expression of CD25, CD11b and APC autofluorescence, and neutrophil CD69 and eosinophil CD18 and PE autofluorescence, but not monocyte HLA-DR and eosinophil CD11a/CD18 ratio, were selected, in addition to the selected SPSS parameters.

Data of 11 test set patients, which were unknown to both classifiers, resulted in a correct recognition of 90% of the patients (SPSS/CLASSIF1: sensitivity, 100%/80%; specificity, 86%/86%; positive prediction, 80%/80%; negative prediction, 100%/85%). These results show that both classifiers are robust for the classification of unknown samples. Misclassification was not assigned to a certain cardiovascular defect or to a certain type of surgery. The coefficients of the unstandardized canonical discriminant function for the SPSS classifier and the CLASSIF1 triple-matrix pattern are provided in Table 4. Using both classifiers (Table 4), classification of the most frequent group of patients from our study (surgery for atrial septal defect closure) resulted in an ARI of 87% for the learning data set (n = 18; sensitivity, 93%; specificity, 100%; positive prediction, 80%; negative prediction, 100%) and in an ARI of

Table 2
24-H Preoperative Surface Antigen Expression on Leukocytes in Patients With and Without POEE[†]

Parameters	Control (n = 28)	Complications (POEE; n = 21)	P value
Monocytes			
CD18	119.7 ± 127.7	226.2 ± 170.0	0.0180*
CD11a	403.7 ± 159.4	718.9 ± 421.0	0.0010*
CD11a/CD18 ratio	5.04 ± 1.83	3.91 ± 1.42	0.0230*
CD45	114.8 ± 88.3	198.1 ± 155.7	0.0254*
HLA-DR	187.0 ± 222.3	574.8 ± 611.5	0.0032*
FITC control	2.60 ± 1.88	3.39 ± 2.03	0.0185**
PerCP control	2.84 ± 1.62	3.64 ± 1.77	0.0070**
Neutrophils			
CD18	78.1 ± 63.7	152.6 ± 115.1	0.0060*
CD11a	142.2 ± 133.1	228.7 ± 140.5	0.0350*
CD11a/CD18 ratio	2.56 ± 1.78	1.54 ± 1.54	0.0042*
CD45	53.5 ± 30.7	84.0 ± 48.3	0.0139*
CD69	4.63 ± 2.43	8.75 ± 4.45	0.0090**
Eosinophils			
Percent of leukocytes	2.33 ± 1.78	3.87 ± 3.12	0.0205**
cells/μl	167.4 ± 147.9	282.2 ± 232.1	0.0480*
CD18	109.6 ± 97.0	182.0 ± 118.3	0.0240*
CD11a	236.5 ± 105.3	356.1 ± 207.7	0.0130**
CD11a/CD18 ratio	3.22 ± 1.43	2.32 ± 1.06	0.0237*
CD45	144.6 ± 93.5	224.2 ± 130.2	0.0203*
PE control	26.9 ± 18.8	38.2 ± 17.9	0.0146**
PerCP control	30.6 ± 19.9	65.3 ± 81.8	0.0110**

[†]If not noted otherwise, parameters are mean fluorescence intensities. Only significantly different values are shown (M ± SD).
*Two-tailed Student's *t*-test.
**Mann-Whitney U-test.

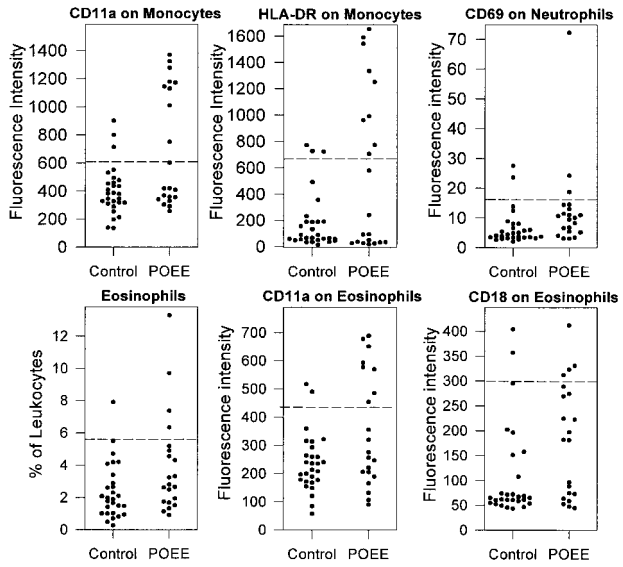

FIG. 2. Antigen expression on leukocytes (fluorescence intensity) and percentage of eosinophils of individual patients without (control) or with POEE. Dashed lines indicate mean + 2 × SD of control. Individuals with values above this line were regarded as at-risk patients.

100% for the test data set (n = 4; sensitivity/specificity/positive prediction/negative prediction, 100%).

Because patient and surgical data for children at risk for developing POEE differed from those of control patients (Table 1), we tested if classification based solely on these data would be comparable to those based on FCM data. However, using SPSS and CLASSIF1, classification based on patient and surgical data alone resulted in a poor discrimination (n = 49; ARI, 76%; sensitivity, 97%; specificity, 55%; positive prediction, 62%; negative prediction, 62%). Classification of patient and surgical data in conjunction with FCM data did not improve the FCM data-based classifier (Tables 3, 4) and none of the patients and surgical data were selected.

DISCUSSION

Children who develop POEE after CPB surgery show significantly elevated cell surface expression of activation markers on monocytes, granulocytes, and eosinophils 24 h prior to surgery. This indicates a preexisting activation of the immune system and permits the preoperative risk assessment by FCM (Table 3). The main discriminators for predicting POEE were elevated activation marker and adhesion molecule expression on monocytes and eosinophils. Although POEE patients were older and were subjected to longer surgery with extended aortic cross-clamping and CPB time, surgical data were not as effective as activation marker and adhesion molecule expression data obtained by FCM in discriminating between controls and patients at risk for developing POEE. These findings suggest that some patients are predisposed to POEE development.

Activated monocytes liberate early the cytokines TNF-α and interleukin (IL)-1 and, together with other factors (13), prime and sensitize neutrophils to the effects of other agonists. Preoperative immune activation may be due to congenital heart disease (14,15) or to subclinical inflammation (9), or it may result from an atopic/allergic predisposition (16,17). The combination of immune activation and CPB surgery as a second stimulus can cause POEE as a summation effect. It is well known that CPB is associated with major qualitative and quantitative leukocyte alterations (23,24,28) as well as with changes of inflammation mediators. A systemic inflammatory response (2,4,5) is generated by interactions among vascular endothelium, platelets, and leukocytes. It is accompanied by signal exchanges, expression of adhesion molecules, and chemokine secretion. CPB causes an increased expression of ICAM-1 (ligand for CD18) on endothelial cells in canine pulmonary capillaries and is associated with an increased accumulation of neutrophils (29).

The most prominent discriminator for POEE prediction was elevated CD11a (LFA–1) expression on monocytes and eosinophils (Table 4). LFA-1 is expressed constitutively on leukocytes. Its expression on eosinophils increases only at an approximately 10× higher chemokine concentration than CD11b (Mac-1) (30) concentrations. CD11a is expressed highly on mononuclear cells and eosinophils of atopic patients (16,17), suggesting a POEE risk for individuals with an allergic/atopic predisposition. This interpretation is sustained by our earlier observation that at-risk patients have elevated levels of serum histamine (19) and IgE (31) and by the finding that a CPB surgery-induced immune response is similar to an allergic reaction (31). A severe allergic reaction may be associated with CPB and cardiac surgery (32) and an allergic predisposition is a risk factor for adverse events after CPB in adults (1). LFA-1 plays a pivotal role in leukocyte adhesion and endothelial transmigration as demonstrated in LFA-1

Table 3
Prediction of Postoperative Outcome of Patients With and Without POEE Based on 24-h Preoperative Data by SPSS and CLASSIF1 Classification

Clinical outcome	n	Predicted control (%)	Predicted complication (POEE; %)
Learning data set			
SPSS			
Control	22	90.9	9.1
Complication (POEE)	15[a]	13.3	86.7
CLASSIF1[b]			
Control	22	100.0	9.1
Complication (POEE)	16	25.0	81.3
Test data set			
SPSS			
Control	6	100.0	0.0
Complication (POEE)	5	20.0	80.0
CLASSIF1[b]			
Control	6	100.0	16.6
Complication (POEE)	5	20.0	80.0

[a]One patient missing due to incomplete data set.
[b]Sum of patients classified exceeds that of all analyzed patients due to double classification of some individuals.

Table 4
*Prediction Parameters and Coefficients of the SPSS and CLASSIF1 Classifier**

SPSS classifier		CLASSIF 1 classifier	
Parameters	Coefficients (c)	Parameters	Change versus control
Monocytes		CD11b	—
CD4	−0.4882	CD4	—
HLA-DR	0.3711		
		CD25	—
		APC control	—
Neutrophils		CD69	+
Eosinophils		CD18	—
CD11a	0.0105	CD11a	—
CD11a/CD18 ratio	−0.0550		
CD45RA	−0.1113	CD45RA	+
PerCP control	−0.0085	PerCP control	+
		PE control	−
(Constant	−2.2250)		

*Calculated based on the learning data set of 22 patients. Parameters are mean fluorescence intensities. Formula of the unstandardized canonical discriminance function (SPSS classifier):

$$\text{Constant} + \sum_{i=1}^{i=6} (p_i * c_i)$$

p_i = measured parameter values, c_i = classifier coefficients. If resulting value < 0 no risk, if >0 POEE risk.

and Mac-1 transgenic mice (33) and in experimental allergic conjunctivitis in mice (34). In vitro blocking of LFA-1 and Mac-1 inhibits neutrophil rolling and adhesion to, as well as monocyte migration through, the endothelium (35,36). Thus, we speculate that leukocytes of patients with elevated preoperative LFA-1 expression attach and transmigrate more easily to the extravasal space while liberating mediators, thus facilitating POEE and possibly CLS (2) as a consequence of additional stimuli.

The potential atopic/allergic generation of POEE susceptibility is an interesting complement to earlier observations, which were suggestive of antecedent infection because of increased CRP (9,29), soluble E-selectin (11), and neutrophil adhesion molecule expression (21), as well as histamine, neopterin, and reduced C1-esterase inhibitor serum levels (19). It is likely that the activated immune system, as a POEE- favoring condition, can be induced by different mechanisms.

Correct classification of most patients was achieved by SPSS and CLASSIF1 by the use of the 6 or 11 most discriminatory parameters of the 122 available parameters. CLASSIF1 provides automated classifications as an advantage for the generation of standardized predictive classifiers. In addition, its direct processing of the original multiparametric FCM data files constitutes an interesting feature for future studies. Exhaustive information extraction and classification are assured even in cases of incomplete data sets (19). CLASSIF1 triple-matrix classifiers are directly comparable during interlaboratory consensus elaboration (20). This avoids the establishment of new learning sets at each hospital and facilitates the interinstitutional use of standardized data pattern classifiers.

Considering the fact that predictions in this context were not possible at all until now, predictive values >80% are promising, especially because FCM can offer much more information. Further information on lymphocytes, antigen expression, antigen ratios, and the spread of the various cell clusters can be explored in future studies using FCM.

Preoperative POEE risk assessment can prevent postoperative complications by starting individualized preoperative therapy (5,34,37). The goal of therapy is the normalization of the activated immune system (1,14–17,32). Predictive classification may also reduce PPS, CLS, MOF, and restenosis in cardiac surgery patients.

ACKNOWLEDGMENTS

We thank Jacqueline Richter for excellent technical help and Peggy Richter for help with the manuscript. The authors thank the Deutsche Stiftung für Herzforschung, Deutsche Herzstiftung (research grant: M.P.) and the Sächsisches Ministerium für Wissenschaft und Kunst (SMWK, research grant: J.B., P.O.) for their support.

LITERATURE CITED

1. Kimmel SE, Sekeres MA, Berlin JA, Ellison N, DiSesa VJ, Strom BL. Risk factors for clinically important adverse events after protamine administration following cardiopulmonary bypass. J Am Coll Cardiol 1998; 32:1916–1922.
2. Tárnok A, Hambsch J, Sack U, Emmrich F, Borte M, Schneider P. Complement, complement activation, and histamine release in children undergoing major operations with and without cardio-pulmonary bypass. Pediatr Cardiol 1999;20:113–125.
3. Picone AL, Lutz CJ, Finck C, Carney D, Gatto LA, Paskanik A, Searles B, Snyder K, Nieman G . Multiple sequential insult cause post-pump syndrome. Ann Thorac Surg 1999;67:978–985.
4. Seghaye MC, Engelhardt W, Grabitz RG, Faymonville ME, Hörnchen H, Messmer BJ, von Bernuth G. Multiple system organ failure after open heart surgery in infants and children. Thorac Cardiovasc Surg 1993;41:49–53.
5. Stieh J, Harding P, Scheewe J, Dütschke P, Kramer HH. Capillary leak

syndrome after open heart surgery for congenital heart defects: therapy with C1-inhibitor. Biomed Prog 1996;9:13–16.
6. Pilz G, Kaab S, Kreuzer E, Werdan K. Evaluation of definitions and parameters for sepsis assessment in patients after cardiac surgery. Infection 1994;22:8–17.
7. Martinez-Alario J, Tuesta ID, Plasencia E, Santana M, Mora ML. Mortality prediction in cardiac patients. Comparative performance of Parsonnet and general severity systems. Circulation 1999;99:2378–2382.
8. Rady MY, Ryan T. Perioperative predictors of extubation failure and the effect on clinical outcome after cardiac surgery. Crit Care Med 1999;27:340–347.
9. Boeken U, Feindt P, Zimmermann N, Kalweit G, Petzold T, Gams E. Increased preoperative C-reactive protein (CRP)-values without signs of an infection and complicated course after cardiopulmonary bypass (CPB)-operations. Eur J Cardiothorac Surg 1998;13:541–545.
10. Hauser GJ, Ben-Ari J, Colvin MP, Dalton HJ, Hertzog JH, Bearb M, Hopkins RA, Walker SM. Interleukin-6 levels in serum and lung lavage fluid of children undergoing open heart surgery correlate with postoperative morbidity. Intensive Care Med 1998;24:481–486.
11. Belch JJ, Shaw JW, Kirk G, McLaren M, Robb R, Maple C, Morse P. The white blood cell adhesion molecule E-selectin predicts restenosis in patients with intermittent claudication undergoing percutaneous transluminal angioplasty. Circulation 1997;95:2027–2031.
12. Munoz R, Laussen PC, Palacio G, Zienko L, Piercey G, Wessel DL. Changes in whole blood lactate levels during cardiopulmonary bypass for surgery for congenital cardiac disease: an early indicator of morbidity and mortality. J Thorac Cardiovasc Surg 2000;119:155–162.
13. Bortolussi R, Rajaraman K, Qing G, Rajaraman R. Fibronectin enhances in vitro lipopolysaccharide priming of polymorphonuclear leukocytes. Blood 1997;89:4182–4189.
14. Marriott JB, Goldman JH, Keeling PJ, Baig MK, Dalgleish AG, McKenna WJ. Abnormal cytokine profiles in patients with idiopathic cardiomyopathy and their asymptomatic relatives. Heart 1996;75:287–290.
15. Kuebler WM, Ying X, Singh B, Issekutz AC, Bhattacharya J. Pressure is proinflammatory in lung venular capillaries. J Clin Invest 1999;104:495–502.
16. Lantero S, Alessandri G, Spallraossa D, Issekutz AC, Bhattacharya J. LFA-1 expression by blood eosinophils is increased in atopic asthmatic children and is involved in eosinophil locomotion. Eur Respir J 1998;12:1094–1098.
17. Tomita K, Tanigawa T, Yajima H, Sano H, Fukutani K, Hitsuda Y, Matsumoto Y, Sasaki T. Expression of adhesion molecules on mononuclear cells from individuals with stable atopic asthma. Clin Exp Allergy 1997;27:664–671.
18. Williams GD, Bratton SL, Riley EC, Ramamoorthy C. Coagulation tests during cardiopulmonary bypass correlate with blood loss in children undergoing cardiac surgery. J Cardiothor Vasc An 1999;13:398–404.
19. Tárnok A, Hambsch J, Borte P, Valet G, Schneider P. Immunological and serological discrimination of children with and without postsurgical capillary leak syndrome. In: Faist E, editor. Proceedings of the 4th international congress on the immune consequences of trauma, shock and sepsis. Bologna, Italy: Monduzzi Editore; 1997. p 845–849.
20. Valet G, Kahle H, Otto F, Bräutigam E, Kestens L. Prediction and precise diagnosis of diseases by data pattern analysis in multiparameter flow cytometry: melanoma, juvenile asthma, HIV infection. In: Darzynkiewicz Z, Robinson JP, Crissman HA, editors. Methods in cell biology. Vol. 64. San Diego, California: Academic Press. 2001;487–508.
21. Inoue T, Sakai Y, Fujito T, Hoshi K, Hayashi T, Takayanagi K, Morooka S. Clinical significance of neutrophil adhesion molecules expression after coronary angioplasty on the development of restenosis. Thromb Haemost 1998;70:54–58.
22. Hodge S, Hodge G, Flower R, Han P. Surface activation markers of T lymphocytes: role in the detection of infection in neonates. Clin Exp Immunol 1998;113:33–38.
23. Galiñanes M, Watson C, Trivedi U, Chambers DJ, Young CP, Venn GE. Differential patterns of neutrophil adhesion molecules during CPB in humans. Circulation 1996;94:364–369.
24. Toft P, Tonnesen E, Zülow I, Nielsen CH, Hokland M. Expression of adhesion and activation molecules on lymphocytes during open-heart surgery with cardiopulmonary bypass. Scand Cardiovasc J 1997;31:91–95.
25. Schmitz G, Rothe G. Durchflusszytometrie in der klinischen Zelldiagnostik. Stuttgart, Germany: Schattauer Verlag; 1994.
26. Gratama JW, D'Hautcourt JL, Mandy F, Rothe G, Barnett D, Janossy G, Papa S, Schmitz G, Lenkei R. Flow cytometric quantitation of immunofluorescence intensity: problems and perspectives. Cytometry 1998;33:166–178.
27. Norussis MJ. SPSS/PC+. Advanced statistics TM. Canyon Lake, TX: SPSS; 1988. p B1–B39.
28. Perttilä J, Salo M, Pirttikangas CD, Jalonen J, Vainio O. Effects of cardiopulmonary bypass on lymphocytes and their subset counts with or without use of autotransfusion devices. J Cardiothor Vasc An 1994;8:532–536.
29. Dreyer WJ, Burns AR, Phillips SC, Lindsey ML, Jackson P, Kukielka GL. Intercellular adhesion molecule-1 regulation in the canine lung after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1998;115:689–698.
30. Jia G-Q, Gonzalo J-A, Hidago A, Wagner D, Cybulsky M, Gutierrez-Ramos JC. Selective eosinophil transendothelial migration triggered by eotaxin via modulation of Mac-1/CAM-1 and VLA-4/VCAM-1 interactions. Int Immunol 1999;11:1–10.
31. Tárnok A, Schneider P. Induction of transient immune suppression and Th1/Th2 disbalance by pediatric cardiac surgery with cardiopulmonary bypass. Clin Appl Immunol Rev 2001;15:291–343.
32. Ecoff SA, Miyahara C, Steward DJ. Severe bronchospasm during cardiopulmonary bypass. Can J Anaesth 1996;43:1244–1248.
33. Ding Z-M, Babensee JE, Simon SI, Lu H, Perrard JL, Bullard DC, Dai XY, Bromley SK, Dustin ML, Entman ML, Smith CW, Ballantyne CM. Relative contribution of LFA-1 and Mac-1 to neutrophil adhesion and migration. J Immunol 1999;163:5029–5038.
34. Whitcup SM, Chan CC, Kozhich AT, Magone MT. Blocking ICAM-1 (CD 54) and LFA-1 (CD11a) inhibits experimental allergic conjunctivitis. Clin Immunol 1999;93:107–113.
35. Gopalan PK, Smith CW, Lu H, Berg EL, McIntire LV, Simon SI. Neutrophil CD18-dependent arrest on intercellular adhesion molecule 1 (ICAM-1) in shear flow can be activated through L-selectin. J Immunol 1997;158:367–375.
36. Shang XZ, Issekutz AC. Enhancement of monocyte transendothelial migration by granulocyte macrophage colony-stimulating factor: requirement for chemoattractant and CD11a/CD18 mechanisms. Eur J Immunol 1999;29:3571–3582.
37. Barnes PJ. Therapeutic strategies for allergic diseases. Nature 1999;402:B31–B38.