prolonged mechanical ventilation) in risk patients undergoing cardiac surgery by standardised monitoring of immunologic parameters.

Patients: From November 2000 until October 2001 we enrolled 94 patients, being either at the age of 70 or more or having a pre-operative left-ventricular ejection fraction <25%, after cardiac surgery with cardiopulmonary bypass.

Methods: Several parameters were measured from the 1st until the 6th (if possible) postoperative day once daily. As two immunocompetence parameters we measured HLA-DR expression on monocytes (antibodies per cell, Quantibrite, Becton Dickinson) and *ex vivo* LPS induced TNF-α production of whole blood (Millenia LPS whole blood assay, DPC Biermann). As proinflammatory parameters we measured PCT plasma levels (Brahms Diagnostika), total levels of IL-8 after lysis of erythrocytes and LBP plasma levels (Immulite, DPC Biermann). Additionally, we also measured plasma levels of the anti-inflammatory cytokine IL-10 (only on day 1 postoperatively).

Results: 24 of the 94 patients developed an infection during the 6 day evaluation period; 8 patients on days 1 or 2 and 16 patients between days 3 and 6. A prolonged mechanical ventilation (more than 24 hours) occured in 21 patients, 11 patients were ventilated more than 48 hours.

All patients showed a post-operative immunodepression which was documented by low monocytic HLA-DR expression and low levels of *ex vivo* TNF-α production after LPS stimulation of whole blood. Patients with early infection (1st or 2nd post-operative day) had lower values in these two parameters compared to patients with late (3rd to 6th postoperative day) or no infection. Total levels of IL-8 were strongly elevated both in patients with early infection and in patients with late infection compared to those patients without infection. Plasma levels of PCT was elevated especially in patients with early infection. IL-10 on day 1 showed higher plasma levels in both patient groups with infection. Elevated plasma levels of IL-10 and PCT on day 1 and high total-IL-8 levels on day 1 lead to an increased relative risk for developing an early or late infection. LBP plasma levels were similarly elevated in all three patient groups without any differences between these groups.

Long-lasting mechanical ventilation occurred particularly in patients with elevated total-IL-8 levels, PCT levels and IL-10 levels on day 1.

There was a small but significant correlation between LPS induced *ex vivo* TNF-α production and monocytic HLA-DR expression as well as a moderate inverse correlation between IL-10 plasma levels and monocytic HLA-DR expression.

Summary: All patients having undergone cardiac surgery with cardiopulmonary bypass showed an immunodepression. Patients with early infection had elevated levels of plasma PCT, total-IL-8 and plasma IL-10 on day 1; increased levels of these three parameters on day 1 indicated the development of infection and also long-lasting ventilation. LBP plasma levels were similarly elevated in all patient groups.

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PREDICTIVE MEDICINE BY CYTOMICS: IDENTIFICATION OF HIGH RISK PATIENTS IN BONE MARROW STEM CELL TRANSPLANTATION

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Goal: The prediction of typical SCT complications like chronic graft versus host disease (GvH) or active CMV infection has the potential to increase overall therapeutic success by early preventive therapy. *Cytomics*, the multimolecular analysis of the cellular heterogeneity in cell systems or organs (*cytomes*) provides a promising approach for individualized disease course predictions. It is based on the assessment of molecular changes at the cellular level of disease development and progression

(http://www.genomicglossaries.com/content/omes.asp).

Methods: Peripheral blood leukocyte 3-color immunophenotypes (FITC/PE/CY5, lyse, no wash) CD3/CD16+56/CD45, TCRab/CD8/CD45, TCRgd/CD4/CD45, CD57/HLA-DR/TCRab, CD8/CD95/TCRab, CD45RO/CD27/CD4, CD45RO/CD62L/CD4 as well as CD8/CD69/TCRab following mAb CD2&2R stimulation for CD69 induction and intracellular IFN-g, IL-2, IL-4, TNF-a, IL-13, CD69 (=xx) as TCRab/xx/CD8 following phorbol ester (PMA)+ionomycin stimulation were analysed by flow cytometry in 80 patients (43 allogeneic/37 autologous) at 2, 3, 6, 9, 12, 18, 24 months post SCT including 117 healthy donors as controls. Cell frequency as well as mean fluorescence intensity, fluorescence intensity ratios and average fluorescence packing density were calculated by quadrant analysis from FSC/SSC autogated lympho-, mono- and granulocyte FITC/PE, FITC/CY5 and PE/CY5 histograms with > 95% of all cells contained in the analysis. The resulting 3.330 data columns were classified (CLASSIF1, http://www.biochem.mpg.de/valet/classif1.html) for *predictive* information with data at 2 months post SCT serving as baseline for the prediction of subsequent clinical complications.

Results: Complication free recovery, chronic GvH, active CMV infection and survival in *allogeneic* SCT was correctly predicted in >95% of the cases at 2 months post SCT. This was also true for the *simultaneous* classification for uncomplicated recovery, GvH, CMV, CMV+GvH. Chronic GvH was predicted by changes in: CD4, CD8, CD45RO, CD62L, CD69, IL2, TCRab, FSC and recurrent CMV infection by changes in: CD3, CD8, CD27, CD45, CD95. The predictions provided complication *indicator* parameters being either increased or decreased in all complications as opposed to complication *discriminators* being differently affected for each complication. Complication indicators were e.g. increased CD4 and CD8 expressions at decreased TCRab and TCRgd positive cell populations. Disease discriminators concerned CD3, CD8, CD1656, CD69, TCRab, HLA-DR, IFNg, FSC. Ultimate non survivors showed increased FSC levels e.g. on IFNg+, IL2+, IL4+, TCRab+, CD69- or HLADR- lymphocytes. *Au-*

tologous non survivors were predictable *prior* to stem cell preparation by discriminators CD62L, CD4, CD8, CD45, CD45RO. This prediction was not correlated to patient's age/sex/CMV/disease status (CLPD, MM, MS).

Conclusion: Complete analysis of the antigenic and light scatter information of peripheral lympho-, mono- and granulocytes provides standardized classifiers for the early identification of SCT risk patients (chronic GvH, CMV, non survival) thus providing a lead time for early preventive therapy. The predictive parameter patterns may be of interest for understanding the cellular pathogenesis of the various clinical complications.

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DETECTION OF ALLO– AND AUTOREACTIVE ANTIBODIES IN FONTAN PATIENTS WITH PROTEIN LOSING ENTEROPATHY (PLE) BY FLOW AND LASER SCANNING CYTOMETRY (LSC).

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Background: PLE is a feared late complication occurring 5-10yrs after Glenn/Fontan type of cardiac surgery with 5–15% of the patients exhibiting a substantial decrease of serum protein by increased secretion of protein. The mortality among patients with a manifest PLE is >60% but its aetiology is yet completely unknown.

Patients and Methods: In a follow up study we analysed patients after Fontan surgery over a period of five years by flow cytometry (FCM) and serology. One patient developed PLE nine months after surgery. The immune sequel was compared to that of seven patients with a manifest PLE, PLE-free Fontan patients and healthy controls.

Result: The immune alterations after PLE are similar for all affected children. It includes the dramatic selective loss of >80% of the circulating alpha, beta T-cell receptor positive CD3+4+ cells. In our essays we tried to find the rational for the selective cell loss. We developed various assays for the FCM and LSC to quantify binding of autoantibodies to cells and tissues. With an FCM based assay we found in the serum of 25% of the PLE patients antibodies binding to leukocytes, especially to T-helper cells. Serum of PLE free Fontan patients and of healthy controls was negative. In 25% of the patients with manifest PLE we found antibodies against intercalated discs by LSC. None of the PLE patients but one Fontan patient without PLE had antinuclear antibodies.

Concussion: We hypothesise that autoimmunity is at least associated with if not participates in the aetiology of PLE.

(Support: Maximilian research award 1997, research grants of Herzkind e.V.).

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USE OF NEAR-INFRARED DYES FOR SLIDE-BASED SIX-COLOR LYMPHOCYTE SUBTYPING

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Introduction: Laser Scanning Cytometry (LSC) is a semi-automated slide-based quantitative technology. Since its commercial introduction many assays for clinical and research use have been established. In addition to a number of fluorescent data that are calculated for every object its exact x- and y-co-ordinate are recorded. This allows to re-localize any cell or object at any time after the analysis and the verify if a cluster of events with distinct fluorescence characteristics consists of debris, artefacts, or cells. Additionally, LSC needs only minimal sample and reagent volumes, it is non-consumptive and non-destructive, and slides can be stored for latter re-analysis.

Material and methods: Initially we have compared immunophenotyping by LSC with the gold standard of flow cytometry. Using 20µl and 100µl, respectively, of peripheral blood from healthy volunteers and from cancer patients we typically found coefficients of correlation of 0.97 to 0.99. For this assay we used a three-colour immunophenotyping (FITC, PE, APC) in combination with a nuclear dye (7-AAD). In order to further increase the capacities of the LSC we changed the filters so that we could detect the novel tandem-dyes PE-Cy7 and APC-Cy7. In order to characterize the detection of these novel dyes we indirectly stained CD3 with the different fluorochromes (FITC, PE, PE-Cy5, PE-Cy7, APC, APC-Cy7) via biotin-streptavidin. We then developed a 6-color assay.

Results: The six-colour assay allows to thoroughly subtype lymphocytes with only one single sample of 10µl peripheral blood by analysing CD3, CD4, CD8, CD19, CD16&56 and CD45. For example, we could take micrographs of the rare subset of $CD3^{neg}CD19^{neg}CD16/56^{neg}$ lymphocytes.

Perspectives: This modification of LSC for the detection of the near-infrared dyes PE-Cy7 and APC-Cy7 could allow to monitor the peripheral immunophenotype in clinical situation which up to date could hardly be analysed at all (neonatal sepsis, intensive care of critically ill infants).

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INCREASE OF CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN PATIENTS WITH CORONARY ARTERY DISEASE AFTER REVERSIBLE EXERCISE-INDUCED ISCHEMIA

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Introduction: The concept of collateral formation in response to tissue ischemia has recently been extended by

Abstracts from the 7th Leipzig Workshop:

"Diagnostic Value of Cell Function Parameters in Cardiosurgical Interventions"

April 12 –13, 2002

Department of Pediatric Cardiology
Cardiac Center Leipzig
University of Leipzig
Germany

Venue: Max-Bürger Forschungszentrum

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