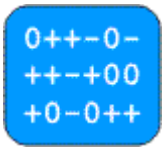

Outcome Prediction in Sepsis Patients by Machine Learning, a Pilot Study

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1. Introduction: Intensive care patients are in a **life threatening** condition when affected by sepsis or non infectious shock, so the prediction of the imminent danger for the development of these states is of high clinical importance.
Clinical **sepsis research** focuses frequently on the determination of **biomarker levels** in patient blood samples. Biomarkers like cytokines are in many instances liberated from immune cells (lympho-/monocytes). These mediators act in part upon effector cells like granulocytes. Depending on immune and effector cell status, given mediator levels may result in stronger or weaker cellular responses, that is *mediator levels are **not directly correlated** with effector cell activities.* **Lymphocytes** defend the organism by cellular and humoral (antibodies) immunity, which typically requires **weeks** to be established while sepsis often develops within **hours**. Like in a medieval city, once the wall is broken (immune defense), the citys fate depends critically on number and activity of intramural soldiers such as **granulocytes** or **monocytes**, acting as important effectors during the elimination of microorganisms or tissue breakdown products by phagocytosis and degradation through oxidative or enzymatic action. The overshooting release of granulocyte enzymes like elastase, of reactive oxygen species like H2O2, O2-, OH. or of pharmacologically active mediators like histamine may **endanger** the organism in case these potent functionalities escape inhibitory control mechanisms like in non infectious shock.
It seemed, therefore, promising to investigate **granulocyte** and **monocyte effector functions** in blood samples of intensive care unit (ICU) patients to determine early outcome predictors for individual ICU patients by data pattern classification.

2. Concept and Goals: Flow cytometric monitoring of bacteria phagocytosis by granulocytes was promising but too complicated to perform in automatated instruments (**CL1**). Cell function assays for the assessment of oxidative burst and proteolytic activities in mono- and granulocytes were therefore developed as an alternative using:
1. humoral stimulators like e.g. cytokines and
2. newly developed sensitive oxidative burst indicator dyes dihydrorhodamine123 (DHR) (8, 10, 11, 14) and specific rhodamine110 substrates for the determination of protease activity (12, 13, 17-22, 24) in vital cells. These developments have substantially simplified the determination of blood cell functions in infection, sepsis or non infectious shock.

3. Results: The early flow cytometric work (1, 2) using bacterial phagocytosis (6, 7), ADB intracellular pH and esterase (1, 2) measurements as well as acridine orange as indicator of cellular and bacterial RNA and DNA(7) had shown for the first time that the **prediction** of imminent danger of sepsis and non infectious shock in **intensive care** (**IC**) patients was possible **two** to **three** days prior to the appearence of life threatening clinical symptoms (**CL1**). Simplified assays using intracellular oxidative burst and proteolytic capacities support these findings (details see below), providing a significantly increased

therapeutic **lead time** for the clinician (CL2 CL3).

4. CLASSIF1 Data Pattern Analysis Flow cytometric data of such measurements are typically collected as list mode files. They are evaluated in a **standardized** and automated way by the CLASSIF1 (CL2 CL3) multiparameter data classification program. The analysis of the entire data set in this way reveiled that the incubation of the blood samples:
- as collected (**ex-vivo status**)
- with **physiological** stimulators such as: suboptimal concentrations of FMLP (*formyl-methionyl-leucyl-phenylalanyl bacterial peptide*), TNF-alpha (*tumor necrosis factor-alpha*), FMLP+TNF-alpha and
- with phorbol ester (PMA, *phorbol-myristate-acetate*) as **maximum** stimulus
(CL2) provides a sufficient amount of predictive information (CL3, **tab.4**) similarly as the determination of **proteolytic enzyme** activities like cysteine or serine proteinases
- The **optimization** of the classification process for the same group of septically admitted IC patients showed that the **most discriminatory** predictive information was contained in the FMLP and TNF-alpha stimulated **oxidative burst** (DHR123) assays (CL3, **tab.8**).
- As a practical consequence of the CLASSIF1 multiparameter data classification, **only two** out of the **seven** performed assays were really required for survival prediction in this group of ICU patients.

5. Conclusions:
A. Functional granulocyte and monocyte parameters provide individualized **predictive** information for the two to three days in advance recognition of life threatening sepsis occurence in ICU patients.
B. The proposed cell assays are suitable for automated preparation, cytometric measurement and standardized data classification.

Literature References:
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