QUANTIFICATION OF INFLAMMATORY MEDIATORS USING THE MULTIPLEX HYBCELL TECHNOLOGY

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BACKGROUND & AIMS

Given the heterogeneity of septic patients, there is a need for point-of-care diagnostic systems to optimise the personalised supportive treatment, such as modulation of inflammatory mediators by extracorporeal therapies.

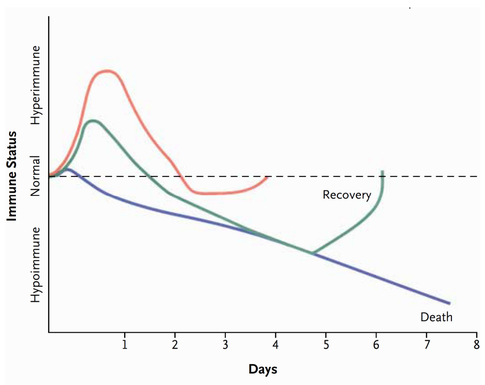

Figure 1. Hyper- and hypoinflammatory phases in sepsis [adapted 1].

Point-of-care diagnostic systems should meet the following needs:

- **proper pattern of inflammatory mediators**
- **small sample volume**
- **rapid detection**
- **easy to handle**

The hybcell technology (Anagnostics Bioanalysis GmbH), based on cylindrical mircroarrays, was established to identify pathogens. Recently, a panel of inflammatory mediators IL-6, IL-8, procalcitonin, C-reactive protein, cystatin C and serum amyloid A has been developed and CE-certified.

The aims of this study were

- **to develop an array for detection of IL-6, IL-8 and IL-10**
- **to compare the sensitivity of cytokine detection between the hybcell technology and conventional ELISA**
- **to assess the influence of anticoagulation on cytokine detection**

METHODS

Development of a Hybcell for Cytokine Detection

A hybcell was developed with Anagnostics Bioanalysis GmbH that can simultaneously measure IL-6, IL-8, IL-10, procalcitonin, C-reactive protein, cystatin C and serum amyloid A. A plasma sample of 100 µl is mixed with a supplied conjugate solution containing competitively labelled proteins and labelled antibodies and applied into the hybcell. Results are available after 20 min.

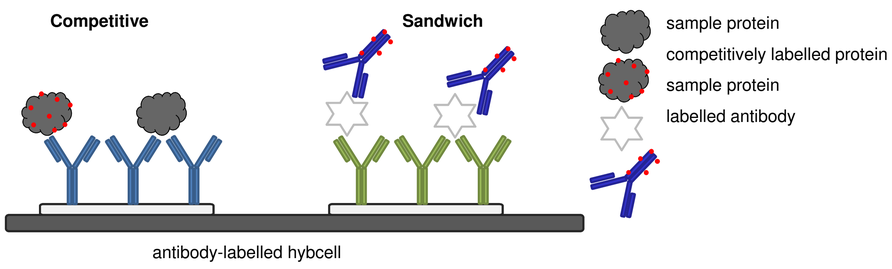

Figure 2. Scheme of hybcell technology.

Sensitivity and Influence of Anticoagulation

Freshly drawn whole blood from healthy donors was anticoagulated either with heparin (5 IU/ml blood), citrate, or EDTA, and incubated with lipopolysaccharide (*E. coli* 1 µg/ml) for 24 hours at 37°C with gentle rocking. After stimulation, blood was centrifuged at 2000xg for 10 min at room temperature to yield plasma containing cell-derived cytokines. Plasma was serially diluted with autologous plasma and cytokines were quantified with hybcells or ELISA (R&D Systems).

RESULTS

Sensitivity

The new hybcell detects IL-6, IL-8, IL-10, procalcitonin, C-reactive protein, cystatin C and serum amyloid A in 100 µl of sample within 20 min. The current detection limit is 200 pg/ml for IL-6 and IL-8 and 500 pg/ml for IL-10.

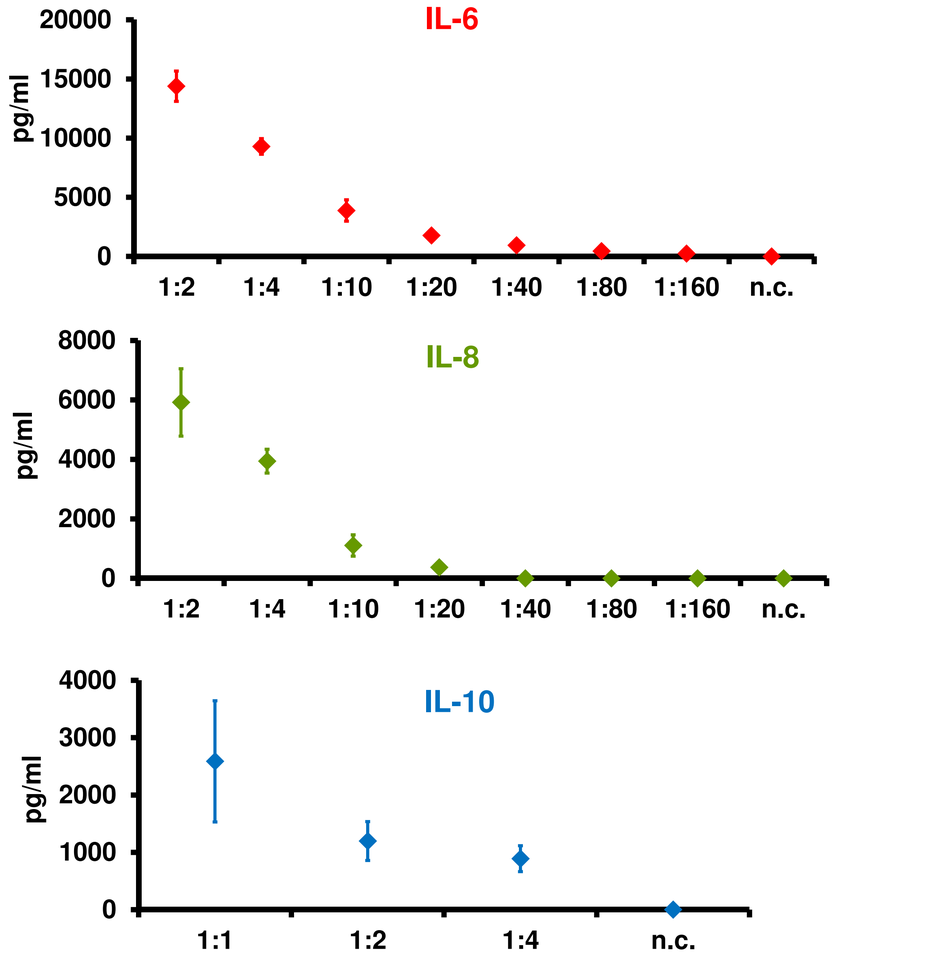

Figure 3. Dilutions of plasma samples containing cell-derived cytokines measured with hybcell technology (n=3, n.c.=negative control).

Hybcell vs. ELISA

Detection of IL-6 and IL-8 is comparable between hybcell and ELISA in citrate- and EDTA-anticoagulated plasma. Cytokine concentrations in heparin-anticoagulated plasma were extremely high and the samples were diluted 100-fold prior to ELISA. Multiplex detection of IL-10 is still under development but shows good correlation to ELISA. As the detection limit is 500 pg/ml with hybcells, the lower concentrations in citrate- and EDTA-anticoagulated plasma could not be detected with hybcells.

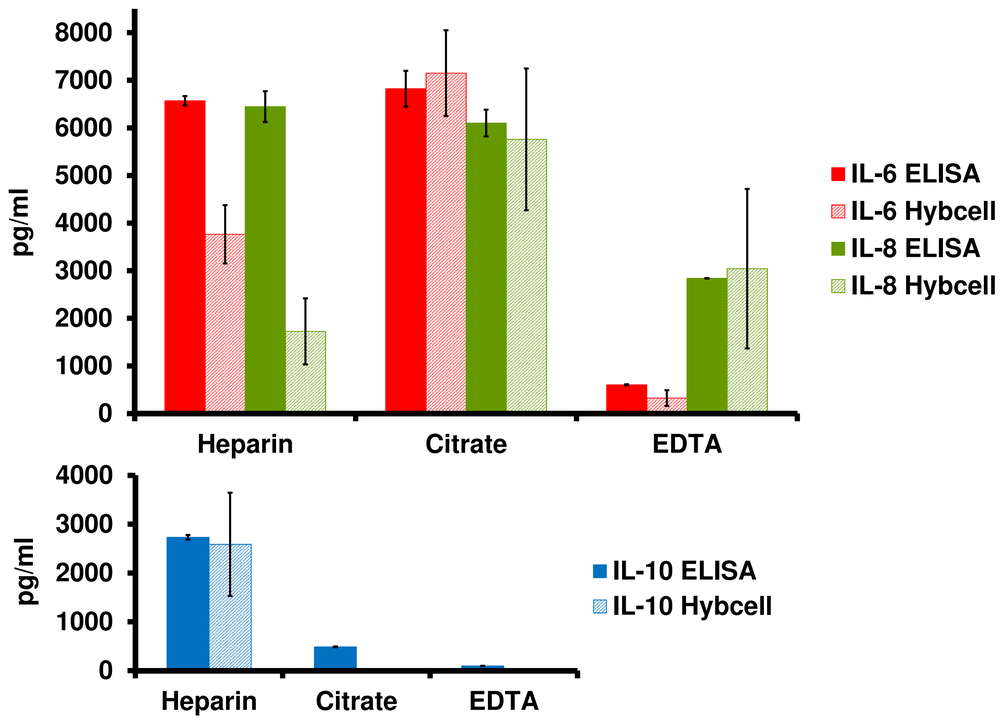

Figure 4. Comparison of cytokine detection in heparin-, citrate-, or EDTA-anticoagulated plasma with hybcell or ELISA (n=3). IL-6 and IL-8 concentrations in heparin-anticoagulated plasma are depicted as 1:15 dilution.

CONCLUSION & OUTLOOK

The hybcell technology allows the simultaneous detection of inflammatory mediators in small sample volumes within 20 min, independent of the anticoagulation method.
The application in clinics, especially for fast point-of-care diagnosis, can be the basis for early onset of adequate therapy and thus better prognosis for patients.

Funding:
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[1] Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med. 2003 Jan 9;348(2):138-50