



Seasonal variation of CRP concentrations in adults studied by a single-step microvolume hsCRP immunoassay based on two-photon excitation of fluorescence

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Abstract

We present a high sensitivity immunoassay concept for C-reactive protein (hsCRP). The single-step microvolume method is based on two-photon excitation of fluorescence and biochemically activated microparticles, the TPX-technology (1-3). The particles act as solid phase carriers for bioaffinity reactions, where covalently coupled primary antibodies bind biomolecules, here CRP, from the solution measured. A labeled secondary antibody, the nanoparticle tracer, is bound to this particle-CRP complex, Fig. 1, and the fluorescence signal from the bound tracer is measured from individual particles by a two-photon excitation fluorometer, directly from the reaction cuvette, without separate washes.

CRP is one of the so-called acute phase proteins, concentrations of which are elevated in response to inflammation, infection and tissue injury. In general clinical practice CRP values over 10 mg/l are considered elevated because of ongoing inflammatory process. However, since elevated CRP concentrations below 10 mg/l are of interest in prediction of future cardiovascular events and stroke, more sensitive methods than usual are required. It has been shown that CRP levels are not subjected to diurnal variation among healthy persons (4).

Our aim was to study the seasonal variation of CRP levels among healthy individuals (n=37) and to determine their CRP baselines from 12-14 consecutive samples (n=470) taken over period of one year.

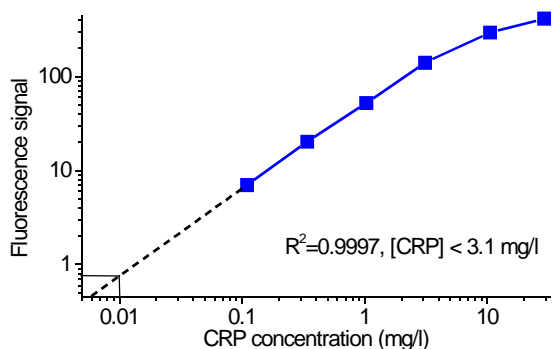


Figure 2. CRP assay standard curve

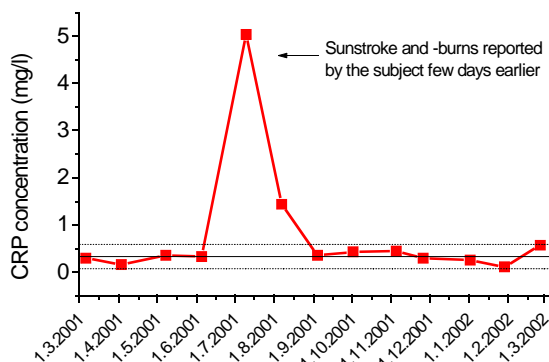


Figure 3. Seasonal variation of CRP in Subject 31. Mean of baseline values (—, 0.33 mg/l) and $\pm 2SD$ (---, 0.26 mg/l).

Assay Protocol

Microparticles: \varnothing 3.22 μm carboxylate-modified latex microparticles from Bangs, coated with monoclonal anti-CRP IgG (Medix Biochemica) by EDC coupling (3). Working dilution 10⁷ particles/ml was prepared in assay buffer.

Nanoparticle tracer: nanoparticles (Orion Diagnostica Oy) were impregnated with BF 560 (Arctic Diagnostics Oy) and coated with polyclonal anti-CRP Fab₂-fragments (4.3 \times 10¹³ particles/ml). Working dilution 1:2000 was prepared in assay buffer.

Assay Reagent: equal volumes of microparticles and nanoparticles were mixed and bath-sonicated in a glass tube for 1 min.

Human CRP Standard Solutions: 0, 0.11, 0.34, 1.03, 3.1, 10.5, 29 (mg/l) (Medix Biochemica) Fig.2

Samples (human serum) and Standards: 1:200 predilutions were made in assay buffer.

Assay: 10 μl premixed assay reagent and 2.5 μl prediluted standard or sample. Incubated overnight in shaker 1000 rpm, at RT. Samples were tested in duplicates.

Measurement: 1 min with a TPX-platereader (Arctic Diagnostics Oy).

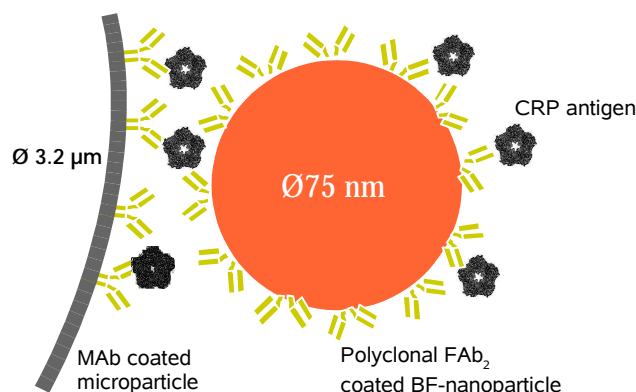


Figure 1. Sandwich type assay concept

Results and Conclusions

Assay detection limit (0-control signal+2SD) was 0.01 mg/l, that is 0.01 $\mu g/l$ in the reaction cuvette. Assay range was 0.01 mg/l-10.5 mg/l. An example of the results is shown in Fig. 3. The individual baseline level was defined by excluding outliers and calculating the mean of those values that were within $\pm 2SD$. Mean baseline level of CRP in the study (n=37) was 0.75 mg/l, SD 0.93 mg/l. Values that were above the subjects' baselines were mainly obtained in the spring and summer, with a peak incidence in May, Fig. 4. There were no statistically significant differences between genders nor between allergic and non-allergic persons.

C-reactive protein seems to be a good marker for evaluating subclinical conditions. However, because of minor trauma, CRP concentrations may fluctuate and lead to seasonal variations. Therefore, it is necessary to take samples periodically and frequently several times to determine a persons baseline.

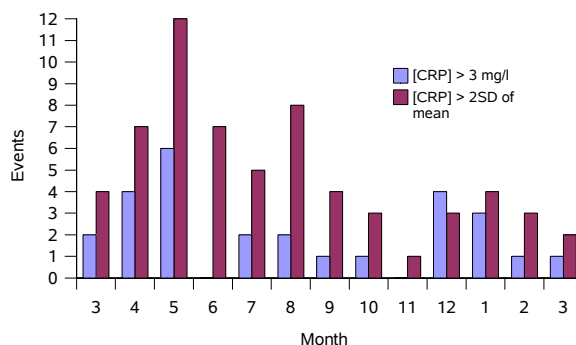


Figure 4. CRP levels were elevated mainly in the spring and summer

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