Nephelometry of Acute-Phase Glycoproteins by Binding to Concanavalin A
Eeva Marja Pitkänen, Timo Palosuo, Kimmo Aho, Tuula Putus-Tlkkanen, and Robert von Essen

Nephelometry of serum acute-phase glycoproteins by binding to concanavalin A (con-A) was compared with assays for haptoglobin and α1-acid glycoprotein, and for C-reactive protein. The cutoff points for positive reactions were determined on the basis of results for a random sample (n = 130) from a middle-aged population. The sensitivity of the con-A binding assay compared favorably with that of individual acute-phase glycoproteins in a follow-up cohort of 198 patients with inflammatory joint diseases. Unlike the case in many individual acute-phase glycoprotein assays, the distribution of con-A binding values in healthy subjects is remarkably symmetrical, allowing an easy distinction between abnormal and normal values.

Additional Keyphrases: haptoglobin · α1-acid glycoprotein · C-reactive protein · cutoff value

Concanavalin A (con-A), a plant lectin, shows an affinity for certain commonly occurring carbohydrate structures that contain terminal glycosyl and mannosyl residues. Most acute-phase proteins in serum are glycoproteins (C-reactive protein and serum amyloid A protein being exceptions) that will bind to, and be precipitated by, con-A. Thus nephelometry of con-A binding has been used to measure acute-phase proteins in serum (1), which have been found to be markedly increased in patients with rheumatoid arthritis (2). The con-A binding correlated well with concentrations of individual acute-phase proteins, both in cross-sectional and longitudinal studies. However, the published data do not allow conclusions as to the sensitivity of the con-A assay in relation to these proteins. Here we compare the sensitivity of the con-A assay with that of assays for two individual acute-phase glycoproteins most commonly used in this context, haptoglobin and α1-acid glycoprotein, and for C-reactive protein.

Materials and Methods

We studied the concentrations of acute-phase glycoproteins and of C-reactive protein in: (a) 130 randomly selected middle-aged (40-59 years) subjects living in a country town in southwestern Finland and serving as controls in an epidemiological study on health effects of inhaled irritants (population series); and (b) 198 adult patients [ages 16 to 77 (mean 41) years] with definite or probable rheumatoid arthritis or nondefinite arthritis, who came for a prescheduled follow-up six to nine years after the onset of their disease (patient series). Patient selection and diagnostic criteria are described elsewhere (3). These patients' disease was generally less active than would be true of patients seeking treatment because of rheumatoid ailments; 53% of them were rheumatoid factor-positive (Waaler-Rose titer ≥92).

In the patient series, the erythrocyte sedimentation rate (ESR; limit for positivity ≥20 mm for the first hour) was used as a nonspecific index of inflammation. We used the extent of clinical involvement of joints as measured by joint score (3) as an index of inflammatory joint disease activity. Joint scores ranging from 3 to 21 were considered as positive.

We used the con-A binding assay originally described by Warren et al. (2) with minor modifications (4), and a Transon 102 FN nephelofluorometer (Orion Analytica, Espoo, Finland). Haptoglobin and α1-acid glycoprotein were determined with the same instrument by equilibrium laser nephelometry, with use of standards and antisera from Behringwerke AG, Marburg, F.R.G. To measure concentrations of C-reactive protein, we used the Behringwerke nephelometry, with a halogen lamp as the light source, standards from Behringwerke, and antisera from Orion Diagnostica. We followed the instrument manufacturer's procedures in measuring the individual proteins.

Results and Discussion

Con-A binding and individual acute-phase proteins were assayed in the population series to determine the cutoff points for positive reactions. The highest 5% of the subjects (seven out of 130) were considered positive. Table 1 shows the frequency of positive reactions in the patient series as a whole, as well as in ESR-positive and joint score-positive subgroups. The positivity was lowest for haptoglobin and highest for con-A binding and C-reactive protein. In evaluating correlations among values for con-A binding and haptoglobin and α1-acid glycoprotein, we found the highest correlation, both in the control population and the patient series, between haptoglobin and α1-acid glycoprotein (0.614 and 0.746, respectively). The lowest in both groups was between con-A and α1-acid glycoprotein (0.508 and 0.639, respectively). Of the patients with a positive con-A binding, 52% exhibited above-normal values for C-reactive protein, and 54% of the patients with above-normal values for C-reactive protein showed increased con-A binding. The C-reactive protein and con-A tests were both positive in 35 of the patients.

The distribution of values for con-A binding in the control population and the patient series, expressed as multiples of the standard deviation from the mean of the population series, is shown in Figure 1. The better to compare patients

<table>
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<th>Table 1. Frequency of Positive Reactions in the Patient Series</th>
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<td><strong>Positive reactions, no. (and %)</strong></td>
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Quantitative cutoff values for positivity were: haptoglobin 3.23 g/L, α1-acid glycoprotein 1.12 g/L, and C-reactive protein 11 mg/L.
with clinical signs of inflammatory joint disease activity with those whose disease was clinically judged to be in remission, we here treated separately subjects with positive (n = 107) and negative (n = 91) joint scores. The distribution of con-A binding values in the population series was remarkably symmetrical, allowing an easy distinction between abnormal and normal values, and increased con-A binding values were found primarily in patients with positive joint scores. The narrow range of con-A binding values in healthy subjects, also pointed out in earlier studies (1, 4), distinguishes this assay from measurements of certain individual acute-phase glycoproteins, which show broader and often skewed distributions.

The con-A binding assay offers further advantages over the measurement of individual acute-phase proteins: no specific antisera are needed, and the system can be used to assess the acute-phase response in humans and in some animal species (7). In addition, the assay is simple and inexpensive, and con-A as a reagent offers uniform quality and well-characterized reactivity with defined ligands. Thus it is well suited for use in epidemiological surveys and as an index of inflammation in experimental studies.

The acute-phase glycoproteins do not respond as rapidly as C-reactive protein and therefore are inferior for evaluating very acute situations, such as bacterial meningitis. On the other hand, the reverse may be true with respect to chronic low-grade inflammatory processes. Compatible with this view, we have previously shown that the con-A assay clearly distinguishes between groups of middle-aged heavy smokers and nonsmokers (4). We assumed that inflammation or infection in the bronchial tree, rather than the smoking itself, was the main reason for the increased con-A binding among heavy smokers. Here we have shown that the sensitivity of the con-A assay compares favorably with that of assays for individual acute-phase glycoproteins. In evaluations of various inflammatory processes, especially when used jointly with C-reactive protein determinations, the con-A binding assay may provide useful information of the acute-phase response.

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References