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Inflammatory activity: Capillary electrophoresis provides more information than erythrocyte sedimentation rate

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ABSTRACT

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Background: A new automated multicapillary zone electrophoresis instrument with improved resolution buffer (Capillarys® with HR buffer, SEBIA, Paris, France) for analysis of human plasma proteins was compared with erythrocyte sedimentation rate (ESR). ESR determinations have been performed for more than eighty years and it is still one of the most frequently used laboratory tests, mainly to monitor the inflammatory response and as a tumour marker. **Methods:** We studied the relationships between ESR, capillary electrophoresis and nephelometric determination of fibrinogen and albumin in 503 consecutive patient samples. The samples were analyzed on the Capillarys®. The albumin concentration from the nephelometric determination was used for quantification of the individual peaks in the capillary electrophoresis electropherogram. **Results:** We found no significant correlation between presence or size of M-components and ESR. There were moderate to strong correlations between ESR, fibrinogen and capillary electrophoretic determination of α_1-antitrypsin, α_1-acid glycoprotein or haptoglobin for the detection of acute phase response. **Conclusions:** We suggest that ESR could be replaced by capillary electrophoresis for the assessment of inflammatory conditions and to detect M-components.

INTRODUCTION

Substantial changes in the levels of several plasma proteins accompany infections or tissue injuries due to trauma, malignancy, or ischemia, as well as inflammatory conditions [1]. The characteristic pattern of these changes is termed the acute phase

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response, which appears to play a significant role in the host defence. The most widely used assays for measurements of the acute phase response are C reactive protein (CRP) [2–5] and the erythrocyte sedimentation rate (ESR) [6–8]. ESR was originally described as a disease marker more than eighty years ago but still retains an important place in medical practice, probably because it is an easily performed and inexpensive test, and a wealth of information about its clinical significance has accumulated over the years. The test measures the distance that erythrocytes have fallen after one hour in a vertical column of anticoagulated blood under the influence of gravity. The accepted upper limits of normal are 15 mm/first hour for males and 20 mm/first hour for females, based on reference values from students in their twenties. Values up to 40 mm/first hour are not uncommon in healthy elderly people [9]. Elevated ESR is a non-specific finding but it is often used as an acute-phase response marker. However, the ESR is influenced by several other factors such as anaemia, erythrocyte size, white blood cell count, immunoglobulins, monoclonal gammopathies, renal failure, pregnancy, age, sex, red cell morphology, room temperature and the placement of the ESR tube [10, 11]. Capillary electrophoresis is an interesting alternative to ESR, as this method can differentiate between M-components and acute phase response and can more accurately measure the increment of individual acute phase proteins including fibrinogen which is the protein that has the largest impact on ESR in the inflammatory response [12, 13]. Any condition that causes a general increase of acute phase proteins (e.g. infectious diseases, polymyalgia rheumatica, temporalis arteritis, diabetes mellitus, heart disease, malignancies, renal failure and pregnancy) will also elevate ESR [13–15]. Capillary electrophoresis systems have been adapted to allow rapid separation of plasma samples. Assay time with the Capillarys® capillary electrophoresis system is less than 10 min. With an automatic interpretation program it should be possible to perform capillary electrophoresis determination of acute phase proteins with shorter turn-around times than ESR, with concomitant determination and quantification of M-components. We were thus interested to study the correlation between ESR performed with Seditainer tubes and capillary electrophoresis for quantification of acute phase response in consecutive patient samples. An automatic ESR reader was used to minimise variations due to interindividual interpretation of the ESR results.

MATERIALS AND METHODS

Patients

The samples (n=503) were from consecutive patients referred to the Department of Clinical Chemistry, University Hospital, Uppsala for ESR determination. The study was approved by the local ethical committee at Uppsala University (01–167).

ESR and nephelometric determination of albumin and fibrinogen

For ESR analysis, blood was collected in Seditainer tubes (366065, Becton Dickinson, Franklin Lakes, NJ USA) and analysed by Sedimatic 100 (Analys Instrument

AB, Bromma, Sweden); normal range 1–20 mm/first hour (< 50 yrs), and 1–30 mm/first hour (> 50 yrs). After ESR analysis, the tubes were centrifugated for 10 min at 1300 g and room temperature. The plasma was transferred to plastic tubes and frozen at –70°C. Plasma fibrinogen and albumin were analysed utilizing a BN Prospec™ nephelometer (Dade Behring, Deerfield, IL, USA) with reagents from the same manufacturer, including a calibrator related to CRM 470. The results were adjusted for the dilution in the Seditainer tubes. All assays were performed at the clinical chemistry laboratory, Uppsala university hospital.

Capillary electrophoresis

Capillary electrophoresis was performed using Capillarys™ Capillary Electrophoresis System (Sebia, Paris, France) utilizing the new HR buffer and the same samples used as for the nephelometric determinations. The instrument is equipped with eight capillaries allowing a throughput of approximately 60 samples per hour. The protein separation is performed at pH 9.9 in silica capillaries and the proteins are detected at an absorbance of 200 nm. The instrument expresses the size of the individual peaks as absorbance percentage of the whole sample. The nephelometric albumin value was used to convert the percentage values for the a1-antitrypsin, α_1-acid glycoprotein and haptoglobin peaks to protein concentrations in g/L.

Statistical analysis

Statistical analysis was performed with Statistica 4.5 (Statsoft Inc., Tulsa, OK, USA) and Excel 97 (Microsoft Corp, Seattle, WA, USA). $P < 0.05$ was considered significant throughout the study.

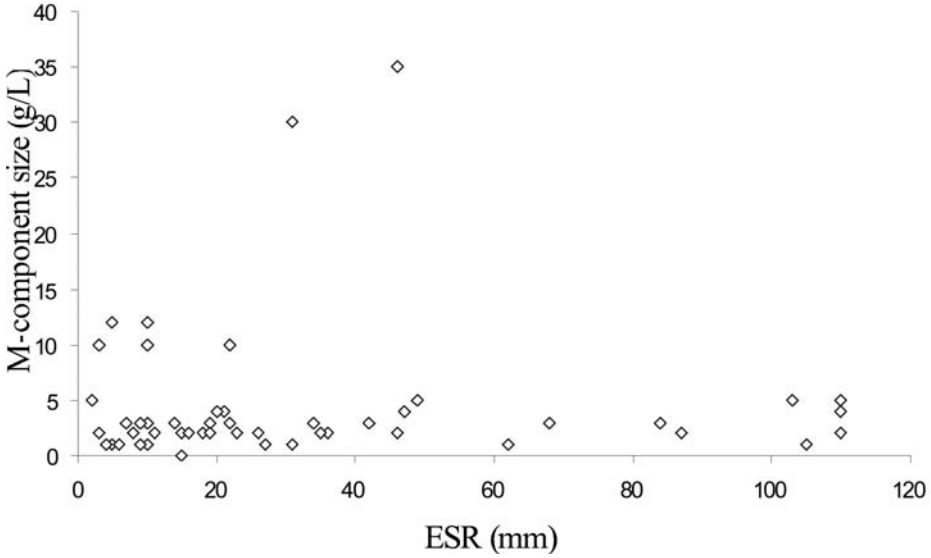

Fig 1. Correlations between ESR and M-component size in samples containing M-components.

RESULTS

ESR

The 503 samples were collected from a total of 538 samples referred to the laboratory for routine ESR analysis. In 35 (6.5%) cases the technician responsible for routine analysis had judged it impossible to perform the assay due to preanalytical errors (insufficient volume or visible clots).

The ESR showed a broad distribution range but with a large group of samples within the normal range. 197 samples (39%) had ESR £10 mm while 74 samples

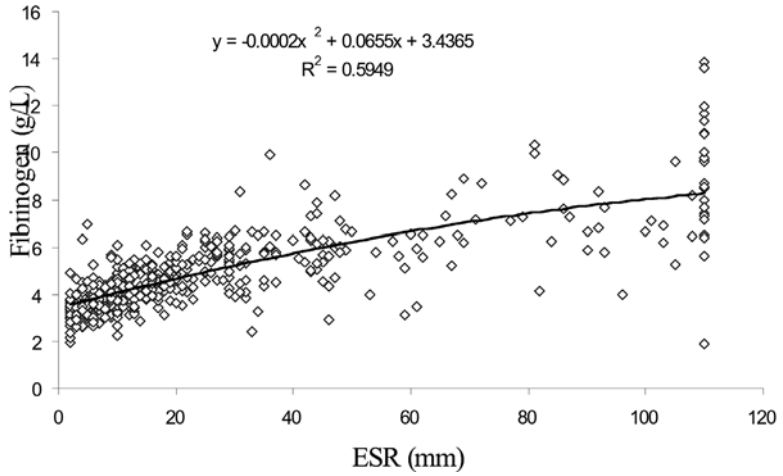

Fig. 2. Correlations between ESR and fibrinogen values in individual patients (n=503).

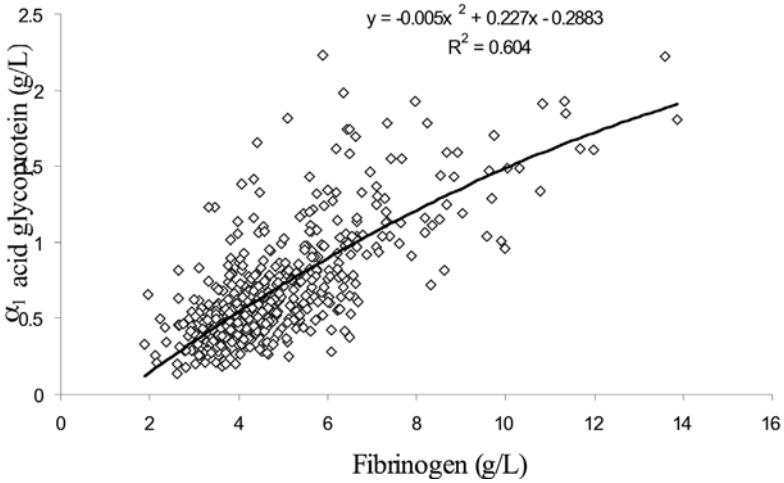

Fig. 3. Correlations between fibrinogen and α_1-acid glycoprotein values in individual patients (n=503).

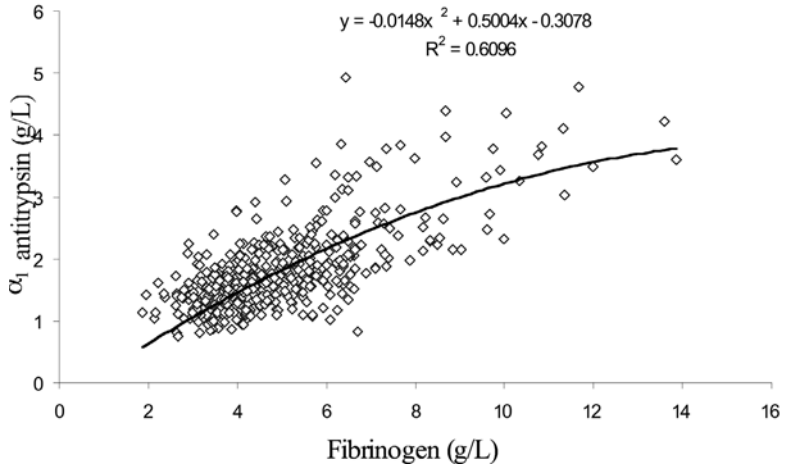

Fig. 4. Correlations between fibrinogen and α_1-antitrypsin values in individual patients (n=503).

(15%) had ESR ≥50 and 27 (5.4%) samples had ESR ≥110. The capillary electrophoresis detected M-components in several of the patient sera. There was no significant correlation between ESR and the presence or size of M-component (Fig. 1.).

Correlations between ESR, fibrinogen and electrophoretic determination of acute phase proteins

There were significant positive correlations between ESR and the other acute phase protein studied: fibrinogen (R^2=0.595), α_1-antitrypsin (R^2=0.437), α_1-acid glycoprotein (R^2=0.451) or haptoglobin (R^2=0.388). The correlation between fibrinogen and the other acute phase proteins measured in g/L was stronger than the corresponding

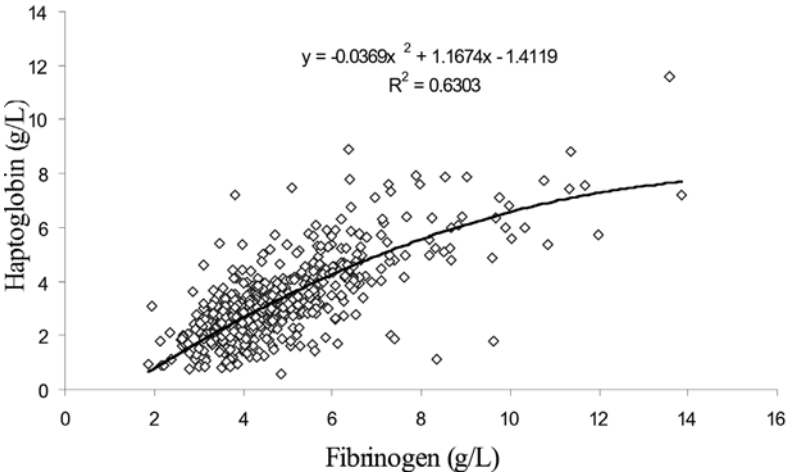

Fig. 5. Correlations between fibrinogen and haptoglobin values in individual patients (n=503).

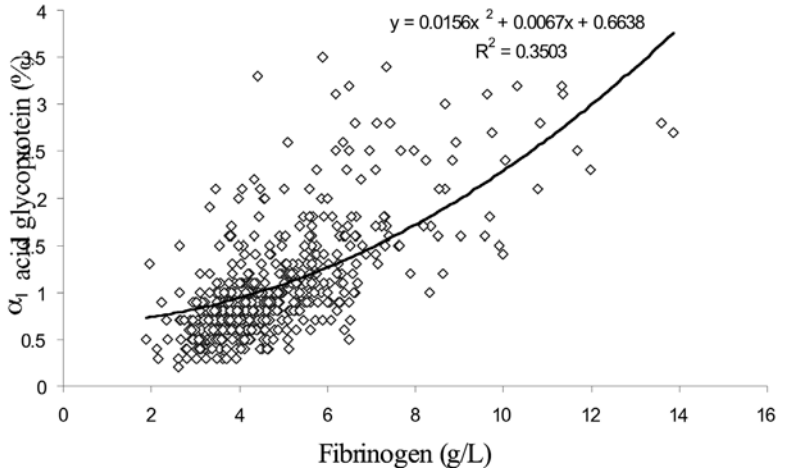

Fig. 6. Correlations between fibrinogen and α_1-acid glycoprotein percentage values in individual patients (n=503).

correlations with ESR: α_1-antitrypsin (R^2=0.610), α_1-acid glycoprotein (R^2=0.604) or haptoglobin (R^2=0.630). If the percentage of the individual peaks from the capillary electrophoresis was used without adjustments for albumin concentration the correlations to fibrinogen were less pronounced: a1-antitrypsin (R^2=0.348), α_1-acid glycoprotein (R^2=0.350) or haptoglobin (R^2=0.363). (Fig. 2–6)

DISCUSSION

The major indications for the measurement of acute phase proteins are to monitor disease activity, and sometimes to provide prognostic information. The tests may also occasionally be used to support a clinical diagnosis [16]. ESR and CRP are the most widely used assays to monitor the laboratory part of the inflammatory process. Currently, ESR is preferred in the Unites States and the CRP is preferred in Europe [17], while fibrinogen and other acute phase proteins are rarely used. In Europe there is a wider use of plasma or serum protein electrophoresis in the southern (e.g. Italy) than in the northern parts. ESR is influenced by several factors unrelated to the inflammation as mentioned in the introduction. During an inflammatory reaction, ESR is mainly influenced by fibrinogen (55%), followed by a2-macroglobulin (27%), immunoglobulin (11%) and albumin (7%) [18]. It usually takes about four to seven days for ESR and fibrinogen to respond, while CRP reacts within one to two days [19]. In chronic diseases like RA it may be an advantage to have a slow and stable inflammatory marker like fibrinogen, a1-antitrypsin, a1-acid glycoprotein or haptoglobin in addition to CRP. There are several factors, other than the acute phase response, that can influence individual plasma proteins. Thus, it may advantageous to determine several acute phase proteins as in capillary electrophoresis. Low α_1-antitrypsin may be due to genetic deficiency while increased levels may be caused by liv-

er damage or oestrogen therapy. α_1-Acid glycoprotein is influenced by the glomerular filtration rate with increased levels in plasma from patients with kidney damage while haptoglobin is low in plasma from patients with liver cirrhosis or haemolysis.

We have previously shown significant correlations between CRP, ESR and fibrinogen in a small group of RA patients [20, 21]. The CRP and fibrinogen, but not ESR, showed highly significant correlation with a functional test (MHAQ) [21]. CRP and fibrinogen have previously been shown to be associated with radiographic evidence of disease progression in RA. There was (at the end of a twelve months period) a better correlation between radiographic progression and CRP than with ESR [22]. Similar results were found in the study by Sjøblom et al., who found good correlation of Larsen index with fibrinogen and CRP, but not with ESR [23].

This study confirms the earlier observations of a correlation between ESR and fibrinogen [12, 20, 21]. Many of the confounding factors of ESR could be avoided using fibrinogen or plasma protein electrophoresis. The many factors influencing ESR makes it difficult to interpret an ESR result. This is probably the cause of the great inter-individual variability in interpretation of ESR results previously reported [24]. In contrast to ESR, capillary electrophoresis can be analysed retrospectively on stored plasma samples and the assay can be centralised, which is important in clinical studies to eliminate interlaboratory variation. The percentage of preanalytical error for ESR (6.5%) is extremely high for a high volume laboratory assay. As insufficient sample and clots have a minor impact on the capillary electrophoresis we also analysed these samples. In a number of these cases, where it was impossible to perform ESR, the capillary electrophoresis revealed the presence of a M-component. The high rate of preanalytical errors is just another argument for the replacement of ESR by more specific assays.

The health care strives to shorten the patient turnaround times (TAT) and thus also laboratory test TAT. The transit from paper and agarose electrophoreses to capillary electrophoresis has made it possible to shorten TAT significantly. The use of lithium-heparin PST tubes eliminates delay due to sample clotting. With PST-tubes, preanalytical automation, capillary electrophoresis, computerised interpretation and an electronic request-result system it should be possible to provide test results within 30 min from the time that the sample arrives to the laboratory. This is superior to ESR TAT. In the future we believe that plasma protein electrophoresis with capillaries will be an interesting alternative to ESR providing shorter turnaround times and more information to the clinician.

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REFERENCES

1. Suffredini AF, Fantuzzi G, Badolato R, Oppenheim JJ, O'Grady NP (1999). New insights into the biology of the acute phase response. J Clin Immunol 19: 203–14.
2. Hedlund P (1947). The appearance of acute phase protein in various diseases. Acta Med Scand 124: 579–82.

3. Mc Conkey B, Crockson RA, Crockson AP (1972). The assessment of rheumatoid arthritis: A study based on measurements of the serum acute-phase reactants. Quart J Med 16: 115–25.
4. Clyne B, Olshaker JS (1999). The C-reactive protein. J Emerg Med 17: 1019–25.
5. Du Clos TW (2000). Function of C-reactive protein. Ann Med 32: 274–8.
6. Fåhreus R (1921). The suspension-stability of the blood. Acta Med Scand 55: 1–228.
7. Kavanaugh A (1999). The role of the laboratory in the evaluation of rheumatic diseases. Clin Cornerstone 2: 11–25.
8. Wollheim FA (2000). Markers of disease in rheumatoid arthritis. Curr Opin Rheumatol 12: 200–05.
9. Shearn MA, Kang IY (1986). Effect of age and sex on the erythrocyte sedimentation rate . J Rheumatol 13: 297–302.
10. Brigden M (1998). The erythrocyte sedimentation rate. Still a helpful test when used judiciously. Postgrad Med 103: 257–62.
11. Zlonis M (1993). The mystique of the erythrocyte sedimentation rate. A reappraisal of one of the oldest laboratory tests still in use. Clinics Lab Med 13: 787–96.
12. Crockson RA, Crockson AP (1974). Relationship of the erythrocyte sedimentation rate to viscosity and plasma proteins in rheumatoid arthritis. Ann Rheum Dis 33: 53–6.
13. Sox HC, Liang MH (1986). The erythrocyte sedimentation rate. Guidelines for rational use. Annals Intern Med 104: 515–23.
14. Brigden ML (1999). Clinical utility of the erythrocyte sedimentation rate. Am Fam Physician 60: 1443–50.
15. Hansson LO, Carlsson I, Hansson E, Hovelius B, Svensson P, Tryding N (1995). Measurement of C-reactive protein and the erythrocyte sedimentation rate in general practice. Scand J Prim Health Care 13: 39–45.
16. Richardson C, Emery P (1996). Laboratory markers of disease activity. J Rheumatol 23: 23–30.
17. Wolfe F (1997). Comparative usefulness of C-reactive protein and erythrocyte sedimentation rate in patients with rheumatoid arthritis. J Rheumatol 24: 1477–85.
18. Stuart J, Whicher JT (1988). Tests for detecting and monitoring the acute phase response. Arch Dis Child 63: 115–17.
19. Blackburn WD (1994). Validity of acute phase proteins as markers of disease activity. J Rheumatol 42: 9–13.
20. Arvidson NG, Gudbjörnsson B, Hällgren R, Larsson, A (1998). Concordant message of different inflammation markers in patients with rheumatoid arthritis. Upsala J Med Sci 103: 35–42.
21. Arvidson NG, Larsson A, Larsen A (2002). Disease activity in rheumatoid arthritis: fibrinogen is superior to the erythrocyte sedimentation rate. Scand J Clin Lab Invest 62: 315–9.
22. Larsen A (1988). The relation of radiographic changes to serum acute-phase proteins and rheumatoid factor in 200 patients with rheumatoid arthritis. Scand J Rheumatol 17: 123–9.
23. Sjøblom KG, Saxne T, Pettersson H, Wollheim FA (1984). Factors related to the progression of joint destruction in rheumatoid arthritis. Scand J Rheumatology 13: 21–27.
24. Thue G, Sandberg S, Fugelli P (1994). The erythrocyte sedimentation rate in general practice: clinical assessment based on case histories. Scand J Clin Lab Invest 54: 291–300.

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