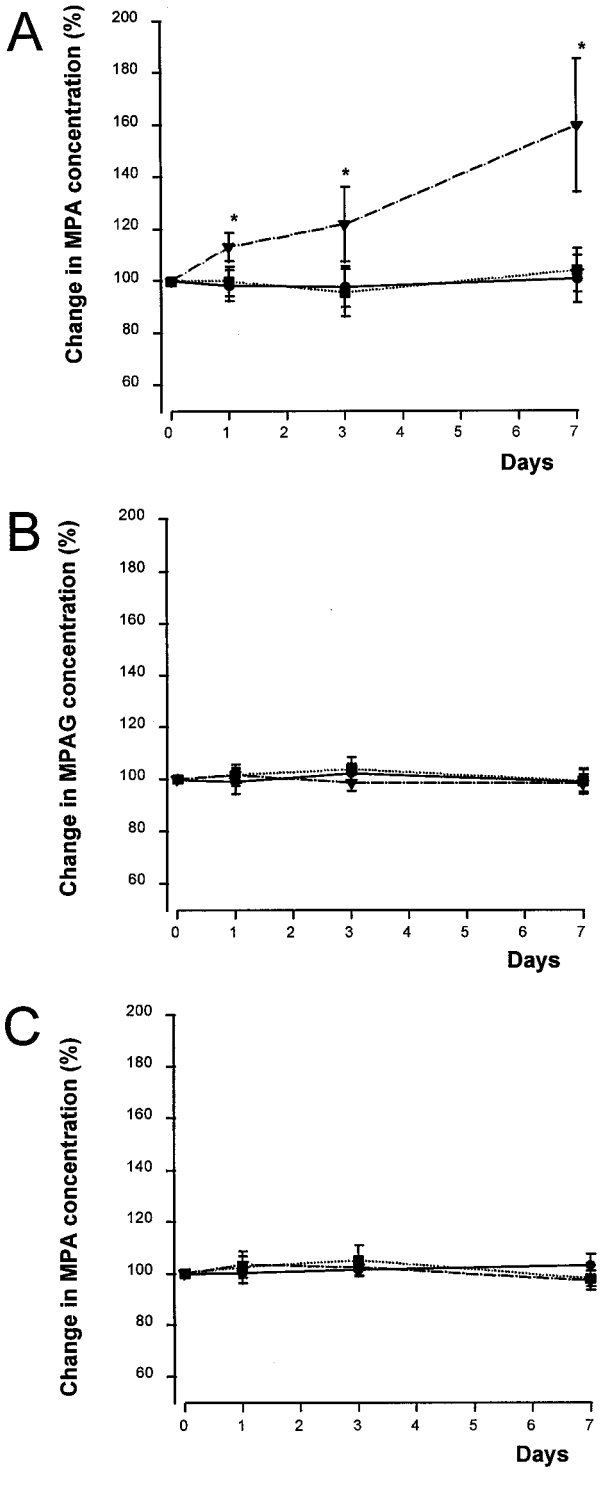

Fig. 1. Overall changes in the concentrations of MPA (*A* and *C*) and MPAG (*B*) in plasma pools stored at −20 °C (●), 4 °C (■), and room temperature (▼).

A, overall changes in the MPA concentrations in plasma pools (n =9) to which MPAG (50, 200, or 500 mg/L) was added. *B*, overall changes in the MPAG concentrations in plasma pools (n = 9) to which MPAG (50, 200, or 500 mg/L) was added. *C*, overall changes (n = 9) in the MPA concentrations in plasma pools to which MPA (1, 10, or 50 mg/L) was added. Data are given as percentages of initial values and are displayed as mean ± SD; ∗, $P < 0.05$. For additional experimental details, see text.

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Interference of Radio-opaque Agents in Clinical Capillary Zone Electrophoresis, *Xavier Bossuyt,* Alex Mewis, and Norbert Blanckaert* (Department of Clinical Pathology, University Hospital Leuven, Kapucijnenvoer 33, B-3000 Leuven, Belgium; * author for correspondence: fax 00 32 16 332896, e-mail xavier.bossuyt@uz.kuleuven.ac.be)

Capillary zone electrophoresis (CZE) has emerged as a novel technique for the rapid and effective separation of serum proteins *(1–8)*. Recently, a multichannel automated system for CZE of human serum proteins (Paragon 2000 clinical capillary electrophoresis system; Beckman Instruments) became commercially available; this system offers a clinically reliable alternative to cellulose acetate and agarose electrophoresis. CZE has the advantage of automation, improved precision, and a faster turnaround time *(6, 8)*.

In the conventional methods, quantification of the protein fractions is based on dye binding, whereas CZE uses ultraviolet detection at 214 nm for direct protein quantification via the peptide bonds. We asked whether intravascular agents such as radio-contrast media that absorb at 214 nm would simulate a monoclonal component on CZE.

We performed high resolution agarose electrophoresis with the Hydrasys analyzer (Sebia), using Hydragel 15 HR gels (Sebia) according to the manufacturer's instructions. The serum proteins were separated into the following fractions: prealbumin, albumin, α_1-antitrypsin, α_1-lipoprotein, haptoglobin plus α_2-macroglobulin, β-lipoprotein, transferrin, complement C3, and the gamma globulins. Spectrophotometric analysis was done with a Varian Cary 3 biospectrophotometer (Varian). We combined sera from five adults for the addition experiments. The capillary zone and agarose electropherograms of each of the five sera were normal.

The CZE electropherograms (Beckman Paragon CZE

2000, Ver. 1.08), determined using conditions as described by Bossuyt et al. *(8)*, from patients who received intravascular radio-contrast medium 2–4 h before specimen collection for electrophoretic analysis are shown in Fig. 1. The radio-opaque media were Urografin® (Schering AG; Fig. 1A), Telebrix® (Guerbet; Fig. 1B), and Omnipaque® (Nycomed Imaging AS; Fig. 1C). In these electropherograms, the α_2-globulin fraction showed a peak ("spike") suggestive of the presence of a monoclonal component. The peak was at the anodal side of the α_2-globulin fraction in the electropherogram for Urografin, in the middle of the α_2 fraction in the electropherogram for Telebrix, and at the cathodal side of the α_2 fraction in the electropherogram for Omnipaque. In each of these cases, however, no peak was discerned on agarose electrophoresis (not shown). The latter observation provided strong evidence against the presence of a monoclonal component.

The unexpected peaks on the CZE electropherograms appeared to represent interference by the radio-contrast agents. First, each of the three contrast agents, Urografin, Telebrix, and Omnipaque, administered to the patients absorbs light at 214 nm (data not shown), which is the wavelength used for quantification of proteins in CZE. Second, no abnormal peak was found when CZE analysis was performed on a specimen collected 2–6 days after intravenous administration of the contrast medium (data not shown). After this time period, the contrast had been cleared from the blood stream. The elimination half-life is 121 min for Iohexol® (the radio-opaque medium in Omnipaque) *(9)*, 120 min for Telebrix, and 60–120 min for Urografin. Third, addition to a serum pool of 7.5 g/L Urografin, 4.7 g/L Telebrix, or 7.5 g/L Omnipaque, concentrations that can be expected after bolus injection for radiographic examination, in each case led to the appearance in the capillary zone electropherograms of abnormal peaks with the same shapes and in the same locations as the extra peaks found in the electropherograms from patients injected with contrast agents. Addition of the contrast agents to a serum pool did not affect electropherograms of samples submitted to agarose electrophoresis (data not shown).

The CZE elution times of the three radio-opaque agents were similar in serum and in 9 g/L NaCl. This indicates that there is no evidence of actual binding of the radio-opaque agents to a specific protein and that the observed interference is purely a result of the coincidence of their elution times and absorption spectra with the elution times and absorption spectra of the proteins.

At our institution, interference by radio-opaque agents is encountered in ~4 of every 1000 specimens submitted for protein electrophoresis. Thus, in high-throughput laboratories, interference by radio-opaque media might be seen in a substantial number of samples.

We conclude that interference by radio-opaque media may be confused with monoclonal proteins and that instructions should be given to not collect blood for protein electrophoresis by CZE shortly after a patient receives contrast media.

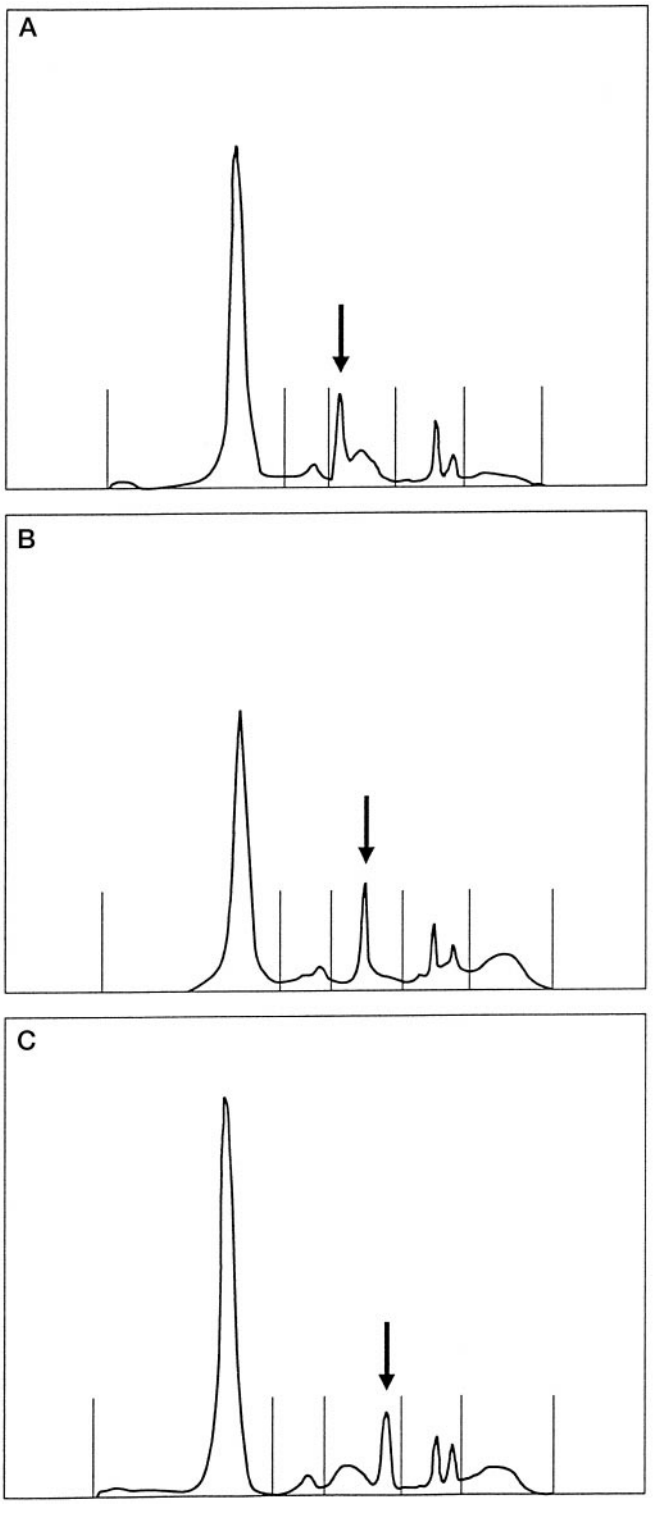

Fig. 1. Effect of Urografin, Telebrix, and Omnipaque on serum electrophoresis.

(*A*) CZE electropherogram of serum collected 2–4 h after the patient underwent perfusion urography with Urografin 30%. (*B* and *C*) CZE electropherograms of serum collected 2–4 h after patients received intravascular Telebrix (*B*) or intravascular Omnipaque (*C*). The peaks for the radio-opaque agents are indicated by *arrows*.

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Absolute or Relative Measurement of Carbohydrate-deficient Transferrin in Serum? Experiences with Three Immunological Assays, *Anders Helander* (Karolinska Institutet, Departments of Clinical Neuroscience and Clinical Chemistry, Alcohol and Drug Dependence Unit at Karolinska Hospital, SE-17176 Stockholm, Sweden; fax 46-8-6721904, e-mail anders.helander@bekl.csso.sll.se)

Individuals who have consumed at least 50–80 g of alcohol per day during the previous week(s) often show an abnormal microheterogeneity of the iron-transporting glycoprotein transferrin in serum *(1)*. After chronic exposure to alcohol, the concentrations of transferrin molecules that lack 2–4 of the four normal terminal sialic acid residues (di-, mono-, and asialo transferrin, respectively) *(1)* or that lack the entire biantennary carbohydrate chain(s) *(2, 3)* increase. The presence of increased concentrations of this "carbohydrate-deficient" transferrin (CDT) is a specific and sensitive biochemical indicator of recent excessive drinking *(1, 4)*.

Whether the CDT result should be expressed as the absolute amount or as the amount normalized to the total transferrin concentration has been a matter of debate *(4)*. The present study compared three commercial immunological test kits for quantification of the abnormal microheterogeneity of serum transferrin observed after excessive drinking: one kit that measures CDT as an absolute amount (CDTect™ RIA; Pharmacia & Upjohn Diagnostics),[1] and two kits that measure the result relative to total transferrin [an RIA (%CDT RIA) and a turbidimetric immunoassay (%CDT TIA), both from Axis Biochemicals].

With the CDTect test, the CDT content is expressed as an absolute amount (in units/L, with 1 unit of CDT in the CDTect assay equivalent to ~1 mg of transferrin) of the transferrin isoforms with a pI ≥5.7. According to a recent report, the CDTect assay measures part of the asialo, monosialo, and disialo isoforms as well as traces of trisialo transferrin *(5)*. In brief, transferrin in the serum sample is saturated with Fe^{3+}, and the isoforms are separated on an anion-exchange chromatography microcolumn. Quantification of CDT is carried out by a double antibody RIA. Because of a gender-based difference in the baseline concentrations of asialo and monosialo transferrin *(6)*, different upper reference limits must be applied for males (20 units/L) and females (27 units/L).

The %CDT RIA test[2] also utilizes an initial ion-exchange microcolumn separation; however, the CDT result is expressed as the percentage of asialo, monosialo, and disialo transferrin relative to total transferrin. This assay apparently measures only a portion of the asialo, monosialo, and disialo transferrin, however, because the upper reference limit of this assay has been defined as 2.5% for both males and females, whereas these isoforms make up ~3% of the total transferrin concentration *(6)*.

The %CDT TIA[3] also measures only a portion (50%) of the trisialo isoform in addition to asialo, monosialo, and disialo transferrin. The CDT result is expressed relative to the total transferrin concentration. The desialylated isoforms eluted from an ion-exchange microcolumn form immune complexes with anti-transferrin antibodies, which are quantified turbidimetrically. The concentration of total transferrin is measured separately, using the same antibodies. An upper reference limit of 6% for both males and females has been recommended by the manufacturer. The intraassay imprecision (CV) of this and the other two methods is <10%.

In this study, serum γ-glutamyltransferase (GGT) activity and total transferrin concentration (traceable to CRM 470) were determined at the local clinical chemistry laboratory, using accredited routine methods.

The same serum sample was used for all analyses, and all tests were done blind to each other and the reference calibrator. Correlations were evaluated through the Spearman coefficient of rank correlation. $P \leq 0.05$ was considered significant. Areas under the ROC *(7)* curves and the significance of differences between areas were calculated using MedCalc software.

Serum samples were collected within 24 h of admission from 239 consecutive patients, ages 17–82 years (94 females, ages 44.6 ± 11.4 years, mean ± SD; and 145 males, ages 47.4 ± 11.2 years) admitted to the addiction treatment center at St. Görans Hospital for detoxification or treatment of alcohol or drug-related problems. Serum was stored at −80 °C until analysis. Information regarding

[1] Axis Biochemicals recently acquired the alcohol-related business from Pharmacia & Upjohn Diagnostics, including the CDTect kit (effective October 1, 1998).

[2] The %CDT RIA kit is no longer commercially available.

[3] Variants of the %CDT TIA kit are also distributed by Roche and Bio-Rad.