With regard to the analysis of our GFC results, Michielsen et al. reanalyze our data and relate elution to their Stokes radius \( R_s \), because they consider elution of nonglobular proteins on GFC to be more closely related to \( R_s \) than to relative molecular mass \( M_r \). Certainly, within the cTnT-I-C complex cTnT is thought to have both a globular domain and an elongated tail region \( (4, 5) \), but the 3-dimensional shape adopted when the protein is present in isolation is unknown. GFC is, like any analytical tool, imperfect, and there are many other variables that influence the elution of proteins in this technique, including the gel, the mobile phase, the protein surface charge, and the hydrophobic domain distribution. The report Michielsen et al. cite in support of their argument concluded that although GFC is not a satisfactory basis for the precise determination of molecular mass, it can be used to obtain an approximation of the relative magnitude of molecular masses \( (6) \). In practice, Wu et al. \( (7) \) have clearly shown that on GFC with Sephacryl S-200 gel (Pharmacia), the cTnT-I-C complex and free cTnT behave as globular proteins and their elution is closely related to \( M_r \). Our results with Sephacryl S-100 gel and the SCIPAC cTn preparation are consistent with those of Wu et al. \( (7) \).

The first peak eluting in Fig. 2D of our article \( (3) \) shows GFC of the SCIPAC material \( (3) \), elutes in a position consistent with the \( M_r \) of the cTnT-I-C complex. We have shown that fractions from this peak react in both the Roche cTnT assay and the Beckman Access cardiac troponin I (cTnI) assay (Fig. 1), supporting our conclusion that this peak represents the cTnT-I-C complex. cTnT reactivity elutes as a shoulder on the leading edge of the peak of cTnI reactivity, suggesting that this cTnT peak represents the cTnT-I-C complex. The cTnI peak contains cTnT-I-C complex poorly resolved from the cTnI complex. Wu et al. \( (7) \) also found the cTnT-I-C and cTnI-C complexes to be poorly resolved. A minor higher-molecular mass form of cTnI, the nature of which we are investigating, is also seen at the void volume. When SCIPAC cTn is subjected to GFC, as in Fig. 2D of our article \( (3) \) and in the Fig. shown here, added cTnT immunoreactivity is quantitatively recovered and none is detected in the void volume, as predicted by Michielsen et al.

On the basis of reanalysis of our GFC results by use of \( R_s \) rather than \( M_r \), Michielsen et al. speculate that the second peak in Fig. 2D may correspond to immunoreactive fragment(s) of cTnT, a circumstance that seems unlikely because Wu et al. \( (7) \) and ourselves \( (3) \) have shown that the increased circulating cTnT immunoreactivity in patients with acute coronary syndrome results largely in elution in the same position, with an \( M_r \) consistent with that of free, intact cTnT.

In their reanalysis of our data, Michielsen et al. calculated \( R_s \) for peaks 1 and 2 to be 37 Å and 26 Å, respectively. Using data from the publication of Wu et al. \( (7) \), we calculated \( R_s \) of 36.9 Å for the cTnT-I-C complex and 27.9 Å for cTnI, in excellent agreement with Michielsen et al. The disagreement over the identity of the peaks in Fig. 2D arises from Michielsen et al.’s interpretation of the relationship between elution and \( R_s \) on GFC. Because primary data are not available, Michielsen et al. have speculated on the \( R_s \) for cTnT and refer to an \( R_s \) value for the cTnT-I-C complex, a value that was determined by use of recombinant, bovine proteins in a different chromatography system and therefore may not be appropriate in the context of our study.

Further work is underway to confirm the identity of the peaks in our GFC system, but practical experience indicates that the elution of the cTnT-I-C complex and free, intact cTnT on GFC using Sephacryl S-100 and S-200 gels is related to \( M_r \), as with globular proteins. We would be happy for Michielsen et al. to subject fractions from our GFC system to their Western blotting analysis. However, we feel that Michielsen et al.’s use of \( R_s \) rather than \( M_r \) in their reanalysis of our data is incorrect, and the conclusions of our study \( (3) \) are valid; cTnT circulates in the free, intact form in patients with kidney failure. Such a conclusion would appear to be more in keeping with the outcomes data that are now emerging, which show increased cTnT concentrations to be powerful predictors of death in both predialysis \( (8) \) and end-stage renal disease \( (9) \) patients.
A Case of Pseudoparaproteinemia on Capillary Zone Electrophoresis Caused by Geloplasma

To the Editor:
Capillary zone electrophoresis (CZE) of serum proteins is a well-accepted method used in clinical chemistry laboratories to separate serum proteins and detect monoclonal (M) proteins. The detection is based on ultraviolet absorbance measurements at 200 nm (Capillaries, Sebia) or at 214 nm (Paragon 2000, Beckman Coulter), which correspond to the absorption of peptide bonds in proteins. Compared with gel electrophoresis, CZE offers the advantage that all proteins are quantified. Most interferences on CZE are caused by exogenous nonprotein substances that also absorb at 200/214 nm, such as radioopaque agents and antibiotics (1). Gelatin-based plasma expanders have also been reported to cause interference. Gay-Bellile and Gijbels reported an increase in the β/γ-region on CZE in samples from patients who had received infusions with 500 mL Gelofusine (2, 3). This interference, however, could not be mistaken for a M-peak.

A 75-year-old woman admitted to the emergency room with symptoms of cardiogenic shock, liver failure, and renal failure was transferred to the coronary care unit. Approximately 24 h later, a serum sample was sent to the laboratory for serum protein electrophoresis. CZE with Capillaries showed an increase in the β/γ-region with a suspected M-peak (Fig. 1A). To further evaluate the unusual morphology on the CZE result, we performed CZE and immunofixation on a plasma sample obtained just before the patient’s transfer to the coronary care unit (Fig. 1B). This sample showed hypoproteinemia (40 g/L) with hypoalbuminemia (18 g/L, 45%), and hypogammaglobulinemia (2 g/L, 6.5%) but no clear M-peak, except for a faint IgG-κ band (Fig. 1B inset). When we compared results from both time points, we observed a 17% decrease in total hemoglobin (from 900 to 750 g/L blood) and a 27% decrease in serum albumin protein (18 to 13 g/L serum), suggesting an increase in extracellular blood volume of ~40%. We contacted the cardiologists and learned that, because of an acute deterioration, the patient had received ~2000–2500 mL Geloplasma (modified gelatin, 30 g/L; Fresenius Kabi) in the 24 h before the sample was taken for CZE. She died 4 h later.

To confirm our observations, we used a hypoalbuminemic serum sample (35 g/L, 47%) that also showed discrete hypogammaglobulinemia (8 g/L, 10%), which we diluted to a total protein content of 40 g/L (Fig. 1C). After mixing the diluted sample with an equal volume of Geloplasma, we observed a similar increase in the β/γ-region and a suspected M-peak (Fig. 1D).

We report for the first time a pseudoparaproteinemia caused by the administration of Geloplasma. Previously, Gay-Bellile et al. (2) observed a polyclonal-like increase in the β/γ-region on CZE in patients treated with Gelofusine. This interference, however, was much less pronounced than what we have observed. In healthy volunteers, approximately half of the Geloplasma is excreted in the urine after 6 h. The interference in the case we report was therefore most likely caused by the administration of large amounts of Geloplasma in combination with renal failure (urea 125 mg/dL; creatinine 211.3 μmol/L, hypoproteinemia, hypoalbuminemia, and hypogammaglobulinemia.

In conclusion, the administration of large amounts of Geloplasma can cause important CZE interference in the β/γ-region, which can be confused with an M-protein in patient samples.

References

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In conclusion, the administration of large amounts of Geloplasma can cause important CZE interference in the β/γ-region, which can be confused with an M-protein in patient samples.

References
Fig. 1. Interference by Geloplasma on capillary zone electrophoresis. (A), electropherogram for serum of a patient who received 2000–2500 mL Geloplasma in 24 h. (B), electropherogram and immunofixation (inset) on plasma of the same patient that was obtained before the administration of Geloplasma. Arrowheads indicate the IgG-Kappa band. (C-D), electropherograms for serum of a patient with hypoalbuminemia and hypogammaglobulinemia diluted to a total protein content of 40 g/L before (C) and after mixing with an equal volume of Geloplasma (D).
MALDI-TOF Mass Spectrometry Compared With Real-Time PCR for Detection of Fetal Cell-Free DNA in Maternal Plasma

To the Editor:
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has recently emerged as a new platform for highly sensitive and accurate analysis of DNA, especially cell-free DNA (cf-DNA) (1–3). In this context MALDI-TOF MS has been shown to permit the detection of paternally inherited single nucleotide base variations from fetal cf-DNA in maternal plasma (2) and donor-derived single-base variations (also known as single-nucleotide polymorphisms) in the urine of kidney transplant recipients (3). Because these were small-scale studies, we conducted a larger study to determine the efficacy of this new methodology, particularly in comparison with the current standard, real-time PCR. Hence, we compared MALDI-TOF MS assay with a well-established TaqMan® real-time PCR assay for the detection of the SRY (sex determining region Y) locus on the Y-chromosome (4).

After we obtained ethics approval from the Cantonal Institutional Review Board of Basel, we collected 97 maternal blood samples from healthy pregnant women (fetal gestational age 7–40 weeks: mean 17.6 weeks). The plasma was separated by centrifugation at 1600g for 10 min, then centrifuged again for 10 min at 16 000g (4). We used 500 μL of plasma for DNA isolation by use of the MagNa Pure DNA isolation instrument (Roche) (5). The cf-DNA was eluted into 100 μL elution buffer, of which 5 μL was used for each assay.

For the detection of the fetal SRY gene sequence by MALDI-TOF MS, we used a Sequenom MassArray® and an assay kindly provided by Sequenom Inc. Briefly, the SRY gene sequence was amplified with the following PCR primers: (forward) 5′-ACG TTG GAT GGT AAC GTT GAC TAC TTG CCC-3′ and (reverse) 5′-ACG TTG GAT GGC CAT TTT TCA GGA CAG CAG-3′. The nonincorporated dNTPs from the amplification products were removed by shrimp alkaline phosphatase. Next, we carried out the homogenous MassEXTEND reaction containing the extension primer 5′-CAG GAC AGC AGT AGA GCA-3′ and the terminator mix of ddGTP, dCTP, dTTP, and dATP. The extended products were cleaned up with SpectroCLEAN resin and dispensed onto a 384-format SpectroCHIP by a MassArray Nanodispenser. The spotted chip was read on a MassArray Analyzer Compact and analyzed with MassArray software (Sequenom Inc). For the TaqMan real-time PCR analysis, the fetal SRY gene sequence was detected with a 7000 Sequence Detector (PerkinElmer Applied Biosystems) as previously described (4). All results of fetal sex determined by these 2 assays were confirmed by clinical data obtained postdelivery.

All the samples were examined in quadruplicate by the MALDI-TOF MS assay and the TaqMan real-time PCR assay in parallel. The presence or absence of the fetal SRY gene sequence was arbitrarily determined as follows: If the fetal SRY gene sequence was detectable in 2 or more of the 4 reactions, the result was scored as positive. Otherwise, it was considered to be negative. Our results indicated that the MALDI-TOF MS assay achieved a sensitivity of 95% (55/58), and the TaqMan real-time PCR assay yielded a sensitivity of 93% (54/58). The specificity of both assays was identical (Table 1). The 3 false-negative cases examined by MALDI-TOF MS assay were also negative according to the TaqMan real-time PCR assay. The 3 samples were obtained from women during early pregnancy (gestational age: 11 weeks, 13.1 weeks, and 13.6 weeks, respectively). The other false-negative result occurring on the TaqMan real-time PCR assay was from a sample obtained at 17 weeks of gestation.

In a comparative analysis, we observed that the MALDI-TOF MS assay and the TaqMan real-time PCR assay had similar detection rates, indicating that MALDI-TOF MS is as sensitive as TaqMan real-time PCR under these conditions. Hence, MALDI-TOF MS may be viewed as an attractive alternative to real-time PCR for the analysis of fetal cf-DNA in maternal plasma.

Table 1. Detection of the fetal SRY gene in cf-DNA from maternal plasma by MALDI-TOF MS and TaqMan real-time PCR assays.

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<th>MALDI-TOF MS</th>
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References