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Short Communication

# Rheumatoid factor interference in the determination of carbohydrate antigen 19-9 (CA 19-9)

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## Abstract

**Background**: Investigation of a 61-year-old Caucasian male suffering from fatigue and weight loss led to the finding of a carbohydrate antigen 19-9 (CA 19-9) concentration of 80 kU/L using an ADVIA Centaur analyser. Determination of CA 19-9 on Vidas, AxSYM and Architect i2000 systems gave normal results. His rheumatoid factor concentration was very high (900 kIU/L) and assay interference was suspected.
**Methods**: Besides using several laboratory procedures to show the cause of the interference, we tried to estimate the frequency of the suspected interference. Therefore, two studies were performed. The first was carried out in a multicentre setting using four different CA 19-9 methods on 51 randomly selected samples with high rheumatoid factor concentrations and ten samples containing no or very low rheumatoid factor. In the second study we used heterophilic blocking tubes for 68 routinely analysed samples with CA 19-9 concentrations ranging between 37 and 250 kU/L using an ADVIA Centaur analyser.
**Results**: In the multicentre study we found eight discrepant CA 19-9 results, but only one was clearly due to interference. We showed that the interference detected, just as in the index case, was caused by rheumatoid factor. The other discrepancies could not be explained, but are probably related to method-dependent differences. In the 68 routinely analysed samples, no interference could be shown using the heterophilic blocking tubes.
**Conclusions**: Although interferences in the CA 19-9 assay are not frequent, the ADVIA Centaur system appears to be more sensitive to rheumatoid factor interference. The lack of standardisation remains an important issue for this assay. The determination of CA 19-9 during the follow-up of patients should be performed using a single method. If, however, there is any clinical doubt about a result, CA 19-9 should be determined using another method to exclude possible interferences.
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**Keywords:** antibodies; artifacts; CA-19-9 antigen; diagnostic errors; false positive reactions; heterophile; rheumatoid factor.

Carbohydrate antigen (CA) 19-9 is frequently used in clinical practice as a tool for staging and follow-up of mainly pancreatic malignancies (1, 2). Although this tumour marker has limited value in the initial diagnosis of pancreatic cancers, determination of CA 19-9 in a 61-year-old Caucasian male suffering from fatigue and weight loss led to the finding of a CA 19-9 concentration of 80 kU/L (cut-off 37 kU/L) using an ADVIA Centaur analyser (Bayer Diagnostics, Tarrytown, NY, USA). In contrast, determination of CA 19-9 on Vidas (bioMérieux, Marcy l'Etoile, France), AxSYM and Architect i2000 systems (Abbott Laboratories, Abbott Park, IL, USA) gave normal results (range 9–19 kU/L) using the same reference level. Pretreatment of the sample using a heterophilic blocking tube (Scantibodies Laboratory, Santee, CA, USA) normalised the result on the Centaur system. Rheumatoid factor (RF) interference was suspected, since RF concentrations were high, at 900 kIU/L. Correlation between the RF and CA 19-9 concentrations on the Centaur system over time is shown in Figure 1.

To evaluate the frequency of this interference in an RF-positive population, we performed a multicentre study in which we determined CA 19-9 concentrations in 51 randomly selected samples with RF concentrations exceeding 100 kIU/L, using the four immunoassay platforms mentioned above. Ten samples containing no or very low RF (<10 kIU/L) were used as controls. RF determination was performed using the Tina-quant RFII kit, a particle-enhanced immunoturbidimetric assay using heat-inactivated human IgG, on a Modular instrument (Roche Diagnostics, Mannheim, Germany). If one of the four methods gave a clinically different result (using a cut-off of 37 kU/L), we considered it discrepant. In these 61 samples, eight samples with discrepant CA 19-9 results were found. These discordant samples were reanalysed, but similar results were obtained. Table 1

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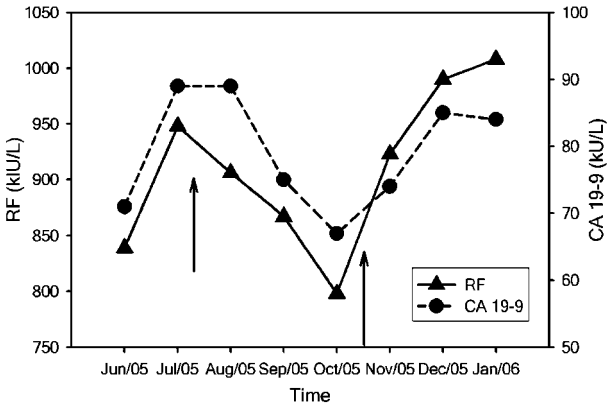


**Figure 1** Concentrations of RF and CA 19-9 over time. Arrows indicate the initiation and cessation of methotrexate therapy. The strong resemblance between the two curves is suggestive of a causal relationship, explaining the interference in the CA 19-9 assay.

shows the results. One sample was from the control group (sample 8). Following pre-treatment of these eight samples with a heterophilic blocking tube, only one sample (sample 1) with a high RF titre (1031 kIU/L) was found, suggesting interference.

We further evaluated our index case and the case found in our multicentre study. These two samples with suspected interference were treated using 25% polyethylene glycol (PEG 6000, Merck, Darmstadt, Germany) in saline solution. The PEG solution (500 μL) was added to 500 μL of serum, vortexed and centrifuged at 1500×*g* for 15 min. Results (taking into account the dilution) were 15 kU/L for both cases (pre-treatment: 80 and 61 kU/L). We also treated the samples with concentrated RF-Absorbant (Dade Behring, Marburg, Germany), which contains sheep IgM antibodies targeted against human IgG-Fc fragments: 500 μL of concentrated RF-Absorbant (final volume 0.5 mL instead of 5 mL) was added to 500 μL of serum, briefly vortexed, incubated for 1 h at room temperature and centrifuged at 1000×*g* for 15 min. Results after this treatment were 9 kU/L for the index case and 15 kU/L for patient 1. We also treated both samples by adding 40 μg of PolyMAK-33 (MAK33-IgG1/IgG1 Poly, Roche Diagnostics) to 250 μL of serum, incubated this mixture for 1 h at room temperature and then centrifuged it for 15 min at 1000×*g*. This also normalised the results. PolyMAK-33 is a polymerised murine IgG1 preparation that is superior in blocking heterophilic antibody activity compared to polyclonal mouse immunoglobulins (3). Finally, a sample from the index patient was treated with 2-mercaptoethanol in saline to a final concentration of 0.05 M. This procedure eliminates IgM from the sample. After incubation for 2 h at 37°C, the result for CA 19-9 was 10 kU/L and RF was 9 kIU/L. This observation is in agreement with the RF isotype: the RF activity in this patient was mainly caused by IgM and only slightly by IgA; no IgG activity could be detected (data not shown).

In the procedures described, two control samples with elevated CA 19-9 concentrations were used to exclude influences on CA 19-9 itself.

Since the RF interference in the two cases described was eliminated with heterophilic blocking tubes, although this is not a ''gold standard'', we used this convenient method to evaluate the likelihood of this specific RF interference in the Centaur CA 19-9 assay. We used heterophilic blocking tubes for 68 routinely analysed samples with CA 19-9 concentrations ranging between 37 and 250 kU/L. The differences between the original measurement and the measurement after treatment with a heterophilic blocking tube were calculated, with results expressed as a percentage of the original result. Results differing by more than 3×SD from the mean difference percentage were considered significant (4). No interference was found in the samples tested.

In 1995, Biguet et al. (5) suggested for the first time the possible interference of RF in the determination of CA 19-9. Our finding of two false-positive CA 19-9 results on the Centaur analyser is, to the best of our knowledge, the only further published observation of this phenomenon. The four CA 19-9 methods employed use the same monoclonal mouse antibody (1116-NS-19-9) as both capture and detection antibody. This could make these assays more susceptible to interferences compared to true ''two-site'' assays using antibodies from different origins. The reason why the Centaur system appears to be more sensitive to RF interference compared to the three other assays could be related to the presence of different blocking agents. Unfortunately, not much information on this matter can be obtained from the diagnostic compa-

**Table 1** Eight samples with discrepant CA 19-9 results from the multicentre study.

| Patient | RF, kIU/L | CA 19-9, kU/L | | | | |
|---|---|---|---|---|---|---|
| | | Centaur | Vidas | AxSYM | Architect | Centaur HBT |
| 1 | 1031 | 61 | 10 | 13 | 14 | 13 |
| 2 | 190 | 42 | 26 | 28 | 26 | 37 |
| 3 | 284 | 47 | 26 | 26 | 53 | 46 |
| 4 | 1100 | 48 | 37 | 38 | 56 | 58 |
| 5 | 647 | 41 | 70 | 70 | 33 | 59 |
| 6 | 371 | 56 | 27 | 32 | 27 | 41 |
| 7 | 1044 | 42 | 23 | 41 | 27 | 42 |
| 8 | <10 | 39 | 16 | 20 | 61 | 46 |

HBT indicates the results on the Centaur system after treatment with a heterophilic blocking tube. Only the sample from patient 1 clearly shows an apparent concentration reduction after HBT treatment, very suggestive of assay interference.

nies. Their protective policies on assay composition are counterproductive when it comes to explaining and anticipating interferences (6).

Although our results are compatible with RF interference, underlying specific human anti-mouse antibodies (HAMA) should be excluded. Both patients with confirmed falsely elevated results (index patient and patient 1) did not have close contact with rodents nor had they ever received any therapeutic or diagnostic products containing mouse antibodies (7). Both samples contained very high levels of RF, but on the other hand, many samples with similar levels did not exhibit this interference, stressing the heterogeneity of RF. RF, as a human anti-human antibody, and heterophilic antibodies, as human anti-animal antibodies, are overlapping entities (8). This explains the interference observed, since only mouse antibodies are used in this assay.

Interferences not only make the CA 19-9 assay sometimes cumbersome; the many discrepant results obtained in our multicentre study, for both RF-positive and -negative samples, are a problem that has previously been addressed (9). Unfortunately, we could not identify the cause of the discrepant results for seven samples, since not enough serum was left for further experiments. The heterophilic blocking tube could not show any interference in these samples. Although this method does not completely exclude all interferences, we believe that the main causes of these discrepancies are method-related differences. The lack of an international standard and the complex nature of CA 19-9 are closely related elements explaining, at least partially, these assay problems (10). Further efforts by diagnostic companies to improve the standardisation of this assay are needed. Until this has been accomplished, follow-up of patients using CA 19-9 measurement should be carried out using a single method. If there is any clinical doubt about a result, CA 19-9 should be determined using another method to exclude possible interferences.

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## References

1. Duffy MJ. CA 19-9 as a marker for gastrointestinal cancers: a review. Ann Clin Biochem 1998;35:364–70.
2. European Group on Tumour Markers. Tumour markers in gastrointestinal cancers – EGTM recommendations. Anticancer Res 1999;19:2811–5. Available from http://egtm.web.med.uni-muenchen.de/index2.html.
3. Reinsberg J. Interferences with two-site immunoassays by human anti-mouse antibodies formed by patients treated with monoclonal antibodies: comparison of different blocking reagents. Clin Chem 1998;44:1742–4.
4. Preissner CM, O'Kane DJ, Singh RJ, Morris JC, Grebe SK. Phantoms in the assay tube: heterophile antibody interferences in serum thyroglobulin assays. J Clin Endocrinol Metab 2003;88:3069–74.
5. Biguet B, Habersetzer F, Beaudonnet A, Bizollon CA, Trepo C, Cohen R. Discordant CA 19.9 serum results by microparticle enzyme immunoassay and immunoradiometric assay. Clin Chem 1995;41:1057–8.
6. Bjerner J, Bormer OP, Nustad K. The war on heterophilic antibody interference. Clin Chem 2005;51:9–11.
7. Levinson SS, Miller JJ. Towards a better understanding of heterophile (and the like) antibody interference with modern immunoassays. Clin Chim Acta 2002;325:1–15.
8. Bjerner J. Human anti-immunoglobulin antibodies interfering in immunometric assays. Scand J Clin Lab Invest 2005;65:349–64.
9. Stern P, Friedecky B, Bartos V, Bezdickova D, Vavrova J, Uhrova J, et al. Comparison of different immunoassays for CA 19-9. Clin Chem Lab Med 2001;39:1278–82.
10. Bechtel B, Wand AJ, Wroblewski K, Koprowski H, Thurin J. Conformational analysis of the tumor-associated carbohydrate antigen 19-9 and its Lea blood group antigen component as related to the specificity of monoclonal antibody CO19-9. J Biol Chem 1990;265:2028–37.

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