PROTEIN PRECIPITATION AS A POSSIBLE IMPORTANT PITFALL IN THE CLINICAL CHEMISTRY ANALYSIS OF BLOOD SAMPLES CONTAINING MONOCLONAL IMMUNOGLOBULINS: 2 CASE REPORTS AND A REVIEW OF THE LITERATURE

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Key words: paraproteins, diagnostic errors, precipitation, interference, artifacts

ABSTRACT

Two case reports are presented, both illustrating an analytical interference caused by monoclonal immunoglobulins. Falsely low results were obtained in the routine analysis of glucose, CRP and HDL-cholesterol.

When analysing samples containing paraproteins, various problems can be encountered in the clinical laboratory: next to the antibody effect, pseudohyponatraemia, hyperviscosity, cryoglobulinaemia and gel formation have to be taken into account. In our two cases the interference was caused by paraprotein precipitation, causing an increased turbidity and an apparent increase of light absorbance at every wavelength due to light scattering, including the wavelengths used in the clinical chemistry assays. We review the literature on this sometimes overlooked interference in photometric/turbidimetric assays. This reaction is based on the insolubility of these proteins in specific physico-chemical circumstances in which many variables are involved, among others: pH and ionic strength, presence of preservatives and surfactants in the assays, pI and other specific properties of the monoclonal immunoglobulins. The complexity of the problem makes predicting or preventing this probably infrequent interference usually impossible. This artifact can cause both false positive and false negative results in multiple parameters (e.g. bilirubin, creatinine, iron, urea, uric acid), the most frequently reported analyte being phosphate.

The Sia water test (Sia euglobulin precipitation test) can provide a first clue to a paraprotein aggregation; confirmation can be obtained by observing the time/absorbance curves of the analysis, performing the test manually or setting up a serial dilution of the sample. The problem can be solved by avoiding the presence of the proteins in the assay, performing the analysis using an alternative method or diluting out the interference.

Both laboratorians and clinicians should be aware of interferences in the clinical laboratory since the clinical consequences could be important.

INTRODUCTION

Monoclonal immunoglobulins, also known as M-proteins or paraproteins (1), can be found in about 1% of subjects over 50 and the prevalence increases with advancing age to more than 3% in persons over 70. They are associated with a wide range of clinical
manifestations, including B-cell lymphoproliferative disorders such as multiple myeloma (Kahler’s disease) and lymphoplasmacytic lymphoma (as in Waldenström’s macroglobulinaemia). Frequently no clinical evidence of a lymphoproliferative disorder can be found, and then the term monoclonal gammopathy of undetermined significance (MGUS) is used (2-5).

Analytical interference in the clinical laboratory is a well-known phenomenon and has many different causes. Both exogenous interferents such as drugs and chemical additives or endogenous substances such as haemoglobin, bilirubin and lipids are well recognized (6-9). Interferences caused by immunoglobulins are more difficult to test or anticipate, but nonetheless they are important (10, 11). Especially immunoassays are sensitive to interferences caused by immunoglobulins (12-14).

Monoclonal immunoglobulins can behave or misbehave just like their polyclonal variants, but sometimes their effect is unusually strong or unpredictable, considering their immunological and physico-chemical homogeneity and their presence in sometimes very high concentrations.

CASE REPORT 1

VC, a Caucasian female born in 1930, had been diagnosed with Waldenström’s macroglobulinaemia in March 1996 and was treated conservatively since then. She was admitted to a peripheral hospital in January 2002 with symptoms associated with blood hyperviscosity: minor mucosal haemorrhages (gums, nose), vertigo with nausea, headache and visual disturbances. Laboratory evaluation was troubled by the high serum viscosity, causing inaccurate sample aspiration and in this way leading to erroneous results. Plasmapheresis was attempted, but failed twice due to the high viscosity. She was initially treated with prednisolone and chlorambucil (Leukeran®), leading to a rapid improvement of the complaints. She was referred to our hospital for further treatment and follow-up. Treatment was continued with cyclophosphamide (Endoxan®), vincristine (Oncovin®), prednisolone. Symptoms improved, oral cyclophosphamide was continued and over time the IgM concentration decreased. In March 2002, at a routine laboratory check-up, we observed a very low serum glucose level (0.2 g/l), measured using a standard hexokinase-based method. Since the sample had been collected approximately 1 hour earlier and the patient had no symptoms suggesting hypoglycaemia, an erroneous result was suspected. Her previous glucose levels had been normal or slightly below the lower reference limit. The glycaemia was checked using a glucose oxidase-based Beckman Glucose Analyzer and a value of 0.8 g/l was found. On the photometric assay, the absorbance of the blank was very high, approaching the absorbance of the end-point. If this very low glucose level had not been encountered, the concomitant important interference in the CRP assay would not have been noticed: we observed a similar pattern in the kinetic curve of the CRP measurement, which yielded the result of <0.1 mg/dl. CRP determination using immunonephelometry gave an apparent value of 4.1 mg/dl, which correlated to the clinical condition since the patient had a chronic leg ulcer. Previous CRP values had always been <0.1 mg/dl. The Sia water test, simply performed by adding a drop of serum to 1 ml of distilled water, was positive: a white flocculate appeared immediately. This simple test is based on the insolubility of some monoclonal proteins in conditions of low ionic strength and offers a clue to the cause of the analytical interference: paraprotein precipitation. IgM monoclonal proteins aggregated when mixed with the sample buffer of the glucose and CRP reagents from our routine, photometry/turbidimetry-based assay. This phenomenon also occurred with other reagent buffers (phosphate, HDL-cholesterol, transferrin), but to a lesser extent and had no influence on the final result. The important interference in the CRP and glucose assays was observed only once more on one of the subsequent visits, but afterwards it disappeared. It has not reappeared yet (February 2004) although the higher background was still seen in the different assays (and the Sia test was positive), but it had no longer a significant effect on the result.

CASE REPORT 2

VNR, a Caucasian male born in 1931, had been diagnosed in May 2001 with an IgG Kappa multiple myeloma, stage Ia according to the Durie-Salmon staging system. On this occasion, total serum protein was 10.9 g/dl and total IgG 5.5 g/dl (measured by immunonephelometry). Chemotherapy was started with oral melphalan (Alkeran®) and methylprednisolon (Medrol®). Three weeks after the start of the therapy, mild leucocytopenia and thrombocytopenia were observed, a slight decrease of the paraprotein levels.
was seen and his initial bone pains had disappeared. The same therapy was continued and 6 courses of chemotheraphy were administered. In May 2002 a thorough re-evaluation showed a serum total protein of 9.4 g/dl, a total IgG of 3.8 g/dl and 36% plasmocytes in his bone marrow. In July 2002 thalidomide was started, but the dose had to be reduced soon due to severe side effects (sedation, tremors, constipation). Low dose thalidomide seemed to induce stability of the disease, but during the year 2003 a slow increase of the paraprotein was observed, leading to IgG levels of 5.1 g/dl in July 2003. Thalidomide therapy was stopped and a new course of oral melphalan/methylprednisolon was started. Initially a good response was observed, but after the second course IgG levels began to rise again, reaching the level of 6.6 g/dl in November 2003. At this time, serum HDL-cholesterol was measured for the first time in this patient and the result from the direct assay was < 1 mg/dl. On the time/absorbance curves of the HDL-cholesterol determination we observed the same phenomenon as described in the previous case: a very high absorbance of the blank. Sia water test was negative in this case. Setting up a serial dilution of the sample in saline resulted in a gradual elimination of the interference, showing that the true HDL-cholesterol was more than 30 mg/dl.

The main difference with the first propositus is that here only a profound effect was seen on the HDL-cholesterol measurement, while no significant interferences with other assays could be found. In contrast to CRP, HDL-cholesterol measurement usually has no important effect on the immediate therapeutic or diagnostic approach to a patient, making this interference less critical. Interference caused by paraprotein precipitation in this new liquid homogeneous HDL-cholesterol assay (15) has been documented (16, 17).

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Analytical interference can be defined as “the effect of a substance present in the sample that alters the correct value of the result, usually expressed as concentration or activity, for an analyte” (8). These purely in vitro artifacts can lead to wrong, missed or delayed diagnoses and unnecessary, potentially harmful interventions (18-20). One has to distinguish an analytical interference from a real alteration of the concentration or activity of an analyte. Paraproteins can bind virtually any antigen, including many autoantigens (21, 22), and can therefore have a clinically important in vivo effect. For example, paraproteins with an inhibitory effect to specific coagulation factors can imply a high bleeding risk and have to be diagnosed as soon as possible (23-25). Not all in vivo effects caused by paraproteins are important, e.g. calcium-binding paraproteins have usually limited physiological consequences (26-28). These paraprotein effects should be distinguished from other tumour related effects, for example in case of Kahler’s disease where secretion of amylase by some myelomas has been described (29-31). Also, one should not jump to conclusions and immediately assume that an observed clinical or laboratory effect is to be attributed to a paraprotein, as was nicely illustrated in a patient with Waldenström’s macroglobulinaemia and a clinically relevant Factor VIII inhibitor, not caused by a monoclonal IgM but by an IgG (32).

Table 1 presents some mechanisms associated with monoclonal immunoglobulins, possibly causing problems in the routine clinical chemistry laboratory. More information on paraproteins causing problems in other fields of the clinical laboratory (haematology, haemostasis and autoimmune serology) can be found elsewhere (11, 23, 24, 41, 42, 53-57). In literature, many papers can be found in which the exact mechanism for interference associated with a paraprotein was not fully elucidated (58-72). As illustrated by our two cases, one of the in vitro interferences caused by paraproteins is protein precipitation, a sometimes forgotten or overlooked cause of spurious results.

PROTEIN PRECIPITATION

We searched the literature for English-language articles published between 1980 and December 2003, using the databases Medline and Science Citation Index Expanded. Table 2 shows the retrieved reports in which protein precipitation has been shown or is very likely to be the main cause of the interference. The most frequently reported assay being affected by this kind of interference is the measurement of inorganic phosphate. A literature review by Larner (87) describes that usually falsely high results are obtained, but also pseudohypophosphataemia has been observed. The author reports incidences of interference ranging...
Table 1. Effects associated with monoclonal antibodies possibly causing problems in the routine clinical chemistry laboratory.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Remarks</th>
<th>Examples and Reviews</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterophilic antibody activity, specific anti-animal antibody activity, rheumatoid factor activity</td>
<td>If blocking agents are available and used, they are probably not always capable of blocking the effect caused by paraproteins.</td>
<td>(12-14)</td>
</tr>
<tr>
<td>Other substance binding (including auto-antigens)</td>
<td>Usually also in vivo, although not always physiologically relevant.</td>
<td>(10, 11, 33-39)</td>
</tr>
<tr>
<td>Agglutinin effect (mainly red cells), especially cold agglutinins</td>
<td>Can cause haemolysis with possible effects on different parameters. Agglutination itself can cause important problems in the field of haematology. Specimen collection, transport, centrifugation and analysis should be at 37°C.</td>
<td>(40-42)</td>
</tr>
<tr>
<td>Cryoglobulin effect</td>
<td>Important problems can also occur in the field of haematology. Specimen collection, transport, centrifugation and analysis should be at 37°C.</td>
<td>(41-44)</td>
</tr>
<tr>
<td>Pseudohyponatraemia</td>
<td>Mainly attributable to the high protein concentrations. Occurs with indirect methods.</td>
<td>(45-47)</td>
</tr>
<tr>
<td>Hyperviscosity effect</td>
<td>Inaccurate sample aspiration.</td>
<td>(48, 49)</td>
</tr>
<tr>
<td>Gel formation</td>
<td>Redissolving the clear gel is usually possible.</td>
<td>(50-52)</td>
</tr>
<tr>
<td>Protein precipitation</td>
<td>See text.</td>
<td></td>
</tr>
</tbody>
</table>

from 10% to 48.6% of the samples. Since this literature review in 1995 two systematic studies have reported incidences of 27% (88) and 19% (89). Another 10 case reports (90-99) have been published on this subject, reflecting its continuing importance.

High concentrations of polyclonal immunoglobulins, as can be seen in inflammatory conditions, liver diseases or auto-immune diseases, have also been reported to give erroneous results in the phosphate measurement (99, 100). Polyclonal immunoglobulins have been reported to interfere with the determination of other parameters as well, such as CRP and ferritin, some of them involving protein precipitation (70, 101). Recently we observed a similar problem as described in our two case reports with the HDL-cholesterol determination in a patient with liver cirrhosis, showing a falsely low HDL-cholesterol concentration of 7.5 mg/dl. The total protein was 8.3 g/dl and on a serum protein electrophoresis a gamma fraction of 59.6% was observed together with beta-gamma bridging.

Mechanisms of precipitation and interference

The exact mechanism of protein precipitation is complex and not fully understood. In order to provide some insight into the mechanism of paraprotein precipitation, we will highlight some key elements of the current model of protein solubility and aggregation.

The charge of a protein is determined mainly by its isoelectric point (pI) and the pH of the solution. The solubility of a protein is usually low when the pH is near its pI (net charge of zero) and the hydration of the protein is at its lowest. In situations where the pH is remote from the pI, more electrostatic charges appear, repulsion forces between the proteins are higher and aggregation is energetically unfavourable. Also, in this situation, salt bridges are formed leading to a higher stability of the protein. In conditions where the pH is far removed from the pI, important electrostatic charges within the protein occur and protein conformation can be affected by these strong repulsive forces, leading to a lower stability of the protein and aggregation as a result (102).

The importance of ions in the solution is illustrated by the phenomenon of salting-out, which is one of the oldest techniques used to isolate proteins from serum or plasma (103, 104). It is induced by bringing proteins in solutions containing high concentrations of salt causing precipitation of the proteins. Opposite to salt-
ing-out is salting-in where the solubility of the proteins is optimised by low concentrations of salt. Although electrolytes modulate the strength of the electrostatic interactions, both within as between the proteins, the predominant effect at low ionic strength is explained by the interaction water-salt-protein. The solubility of a protein is the result of an interaction between ionisable groups from the protein and ions from the salt in solution. At least as important as the salt concentration, is the salt composition. The degree of salting-in and salting-out according to the composition of the salt is described by the lyotropic series, also called Hofmeister series. High-lyotropic-series salts contain small ions of high charge density (kosmotropes), for example Li⁺, F⁻ and SO₄²⁻, and bind water molecules strongly relative to the strength of water-water interactions. The water molecules surrounding these ions compete for the water surrounding the proteins and are very effective in salting-out. Low-lyotropic-series salts contain large monovalent ions of low charge density (chaotropes), for example I⁻ and Br⁻, bind water molecules more weakly than water itself, can themselves interact with the protein and are therefore well suited for salting-in, but have very low capacity of precipitating proteins (105).

Furthermore, interactions between pH and salts, protein stabilising effects of high concentrations of kosmotropes and protein destabilising effects of high concentrations of chaotropes should be taken into account (106).

Routine clinical chemistry assays are constructed in such a way that most proteins, including normal immunoglobulins, do not interfere. Even if an immunoglobulin derived from a normal single cell would precipitate in a certain assay, this would have no consequences since the concentration of this protein is extremely low. If this cell should clonally proliferate and enough of the monoclonal immunoglobulin would be produced (enough for that specific assay for that specific para-

**Table 2. Assays reported since 1980 in which paraprotein precipitation is likely or definitely the cause of interference.**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Effect: falsely...</th>
<th>Paraprotein Isotypes (cases/tested)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>Low</td>
<td>IgM (1/1)</td>
<td>(73)</td>
</tr>
<tr>
<td>Bilirubin (Total)</td>
<td>High</td>
<td>IgG (1/1), IgM (1/1)</td>
<td>(74)</td>
</tr>
<tr>
<td>Calcium</td>
<td>High</td>
<td>IgM (2/2)</td>
<td>(75)</td>
</tr>
<tr>
<td>Chloride</td>
<td>High</td>
<td>Not mentioned (3/5)</td>
<td>(76)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>High</td>
<td>IgM (3/3)</td>
<td>(77)</td>
</tr>
<tr>
<td>Glucose</td>
<td>Low</td>
<td>IgM (2/2)</td>
<td>(78)</td>
</tr>
<tr>
<td>HDL-Cholesterol</td>
<td>Low</td>
<td>IgM (2/6), IgG (2/6), IgA (0/1)</td>
<td>(16)</td>
</tr>
<tr>
<td>HDL-Cholesterol</td>
<td>Low</td>
<td>IgM (1), IgG (1), polyclonal IgG (1) / 15 tested in total</td>
<td>(17)</td>
</tr>
<tr>
<td>Iron</td>
<td>High and Low</td>
<td>IgA (2/8), IgG (13/38), IgM (3/10), IgD (0/2)</td>
<td>(79)</td>
</tr>
<tr>
<td>Iron</td>
<td>High</td>
<td>IgG (1/1)</td>
<td>(80)</td>
</tr>
<tr>
<td>Iron</td>
<td>High</td>
<td>IgG (3/3)</td>
<td>(81)</td>
</tr>
<tr>
<td>Phosphate</td>
<td></td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>Protein (Total)</td>
<td>Low</td>
<td>IgM (3/3)</td>
<td>(82)</td>
</tr>
<tr>
<td>Protein (Total)</td>
<td>Low</td>
<td>IgM (10/10), IgG (3), IgA (1) / 18 myelomas tested</td>
<td>(83)</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>Low</td>
<td>IgG (3/3)</td>
<td>(84)</td>
</tr>
<tr>
<td>Urea</td>
<td>High</td>
<td>IgG (9), IgA (1) / 34 tested in total</td>
<td>(85)</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>Low</td>
<td>IgM (7/11), IgA (0/9), IgG (0/11)</td>
<td>(86)</td>
</tr>
</tbody>
</table>

* Studies used water as blank/diluent!
protein), the precipitation could become relevant and influence the assay.

Precipitation of paraproteins in clinical laboratory assays mainly interferes with assays using a photometric detection. It causes a turbidity, which apparently causes increased light absorbance at every wavelength, including the wavelengths used in the specific monitoring of the reaction. Depending on the time of the appearance of the aggregation (e.g. blanking, addition of first or second reagent...), falsely high or low results can be found.

Prevention of precipitation?

Guidelines and protocols exist for the evaluation of different kinds of interferences in clinical chemistry assays (107, 108), but paraprotein precipitation is almost impossible to exclude (86, 109) considering 1) the solubility of proteins being dependent on pH and salts,
2) the wide variation in pH, electrolyte concentrations and composition of assays,
3) the wide interindividual variation in concentrations and pI's of paraproteins,
4) other factors such as temperature, presence of preservatives, surfactants,... can play a role in the solubility of proteins (102, 110).

Although in an individual patient the concentration of a monoclonal antibody can be correlated to the degree of interference, systematic studies show that nor the type, subclass, isoelectric point or the concentration of the monoclonal immunoglobulin are predictive of a possible precipitation (16, 74, 79, 80, 88, 89, 110, 111). Therefore the overall incidence of this specific interference is very difficult to estimate, but is for most assays probably low.

Blanks based on water are more prone to precipitation and the introduction of saline or buffer based blanks has solved this problem partially (78, 83, 86). Using dichromatic analysis or using kinetic measurements (instead of one or two point measurements) can also sometimes prevent this kind of interference (112).

Modern laboratory techniques have enabled a high throughput of samples while maintaining or improving overall quality of the analyses, and at the same time reducing costs (113, 114). Problems such as protein precipitation, which were once considered every day practice but which are now rarely encountered, remain nonetheless important. The presently available methods should enable us to detect interferences if we use their full capabilities. Some automated analysers can detect the presence of haemolysis, bilirubinaemia or lipoaemia. An alert for the possibility of an interference could be a simultaneous flag for these three parameters, without the sample being visually icteric, lipoaemic or haemolytic (78, 115). One could imagine current laboratory structure and information systems able of producing a warning when analysing a sample containing a paraprotein, as concluded from a serum protein electrophoresis, in this way demanding extra attention from a laboratory technologist.

RECOGNIZING PARAPROTEIN INTERFERENCES IN THE LABORATORY

The first clue to an analytical interference is usually a clinically impossible or improbable result, although only minor errors can occur which are therefore usually not noticed by the lab and also often not considered by the clinicians.

Each laboratory has its specific ways of dealing with possible interferences. It usually implies checking other and previous results of the patient (delta-check), repeating the assay, checking the sample for haemolytic, icteric or lipoaemic aspect, high viscosity and the presence of clots. Preanalytical problems such as a very difficult or wrong specimen collection (e.g. contamination from infusion), wrong collection tube and incorrect transport or storage of the sample have to be considered. These first steps will result in identifying the most frequent errors. If the inconsistency remains, other interferences, especially drugs or other substance interferences, including therapeutically or diagnostically administered products, have to be considered (42, 116, 117). Subsequent steps will be checking the reaction curves for high background or abnormal reactions, gathering clinical information about the patient, searching for information on interferences supplied by the manufacturer or other sources (6, 7). Setting up a serial dilution series of the sample and finding a non-linear correlation in the observed results, is a clue for an interference (14, 73, 90).

Interference in immunoassays is somewhat special since antibodies are used as a reagent. Some specific interferences can be present in these assays: heterophilic antibodies, specific human anti-animal antibodies, rheumatoid factors. Commercial blocking reagents to these specific antibodies are available and some are routinely applied (5, 12, 13). Paraproteins can induce all those specific interferences, but the sometimes high
concentrations can make the classic problem solving methods inadequate. Next to the specific antibody capacity of monoclonal immunoglobulins, precipitation must not be forgotten.

Although any paraprotein can cause precipitation, IgM has been reported to be frequently involved (110). More systematic studies not using water/serum as a blank, do not appear to confirm this impression (79, 88, 89, 118, 119). A classical test called the Sia water test (Sia euglobulin precipitation test), named after its originator and first used in the differentiation between kala-azar and malaria, was later on modified and used as a screening test for Waldenström’s macroglobulinaemia (120, 121). Euglobulins such as IgM are proteins that precipitate at neutral pH (e.g. water without electrolytes). Adding a drop of serum containing an IgM paraprotein to about 1 ml of distilled water can produce a white flocculate at once (73, 115, 120). Performing this cheap and simple test provides an immediate clue to paraprotein precipitation, but it has a low specificity and no complete sensitivity for a possible interference since the physico-chemical circumstances in clinical chemistry assays are considerably different from water. Although high backgrounds or abnormal reactions in the reaction curves can learn a lot about a possible interference, visual inspection of the reaction cuvette or performing the test manually allows the observation of precipitate formation.

PRACTICAL SOLUTIONS TO THE PROBLEM

The easiest way to solve problems with method specific interferences is of course to perform the analysis by using another method. In paraprotein precipitation, specific physico-chemical conditions are responsible for the interference and simply changing a buffer or introducing other minor modifications in the assay can be sufficient for avoiding the precipitation (79, 119, 122). One has to be certain not to change the assay performance when modifying the composition of the reagents. Diluting out the interfering substance, in case paraproteins, can be a clue as well as a solution to an interference: diluting the sample maximally until the interference is not present anymore. Obviously the dilution cannot continue everlasting since also the analyte one wants to determine is diluted. Maybe this is not a very accurate method, however it can give an estimate of the true value if no other methods are available and in cases where a semi-quantitative measure is adequate (79, 80, 119). Selectively removing the paraprotein from the serum can be a solution, but techniques as ultrafiltration, ultracentrifugation, dialysis, immunoabsorption or adding dithiothreitol are not always routinely available or applicable. An easy and cheap method to deal with paraprotein interference is using precipitation techniques, but one has to make sure that the compound of interest is insensitive to this procedure (65, 90, 97, 104, 123, 124).

Some final suggestions when having encountered an interference:
• Check all the other performed analyses on the sample since multiple parameters could be influenced by the interference and some could remain unnoticed!
• Analytical methods that use a kind of physical barrier (including dry chemistry methods) do not experience protein precipitation problems and can therefore be an alternative when analysing these samples (125).
• Other parameters or using urine as a sample can possibly provide you with some relevant information required to treat a patient (e.g. urea vs. creatinine).
• Using a normal sample as a control is a good method for checking possible alterations when deproteinising or modifying a method to get rid of the interference.
• Treatment of the patient leading to lower levels of the paraprotein can also solve the problem.

CONCLUSIONS

Since systemic deproteinisation of serum is no longer applied in modern routine clinical chemistry assays, interferences caused by paraproteins can occur more often. These unpredictable interferences can give both false positive and false negative results and can go by unnoticed to the laboratory. Paraprotein precipitation, being one specific kind of interference, is sometimes overlooked, both in daily practice as in literature. Optical techniques applied in an aqueous environment could be sensitive to this specific interference. Clinicians should be aware of these aspects of paraproteins and contacting the laboratory should be encouraged when encountering unexpected results in patients harbouring a monoclonal antibody. In the laboratory, care should be taken to either circumvent these problems by using techniques less sensitive to
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the problem or one could implement a warning system, drawing extra attention to a sample harbouring a para-
protein.

SAMENVATTING

Aan de hand van twee patiënten illustreerden we het belang van analytische interferentie veroorzaakt door paraproteïnen in routine biochemische analyses. In deze patiënten vonden we vals verlaagde resultaten voor glucose, CRP en HDL-cholesterol.

Verscheidene problemen kunnen zich voordoen wanneer we in het klinisch laboratorium monsters analyseren die monoklonale eiwitten bevatten. Naast hun specifieke antilichaam eigenschappen, moeten we ook bedacht zijn op pseudohyonapronie, hyperviscositeit, cryoglobulinemie en gelvorming. In de voorgestelde casussen was het probleem echter eiwitprecipitatie, waardoor een verhoogde turbiditeit ontstond en een schijnbaar verhoogde lichtabsorptie optrad in de meetmethode. We hebben de literatuur rondom deze, bij elkaar regelmatig miskende, interferentie nagekeken. Het neerslaan van deze eiwitten in waterig milieu is afhankelijk van verschillende factoren, onder meer de pH en de ionsterkte, de aanwezigheid van bewaarmiddelen of detergenten in de reagentia, de pl en andere specifieke eigenschappen van de eiwitten. Het is dan ook quasi onmogelijk om een dergelijke, vermoedelijk niet frequente, interferentie te voorzien of te vermijden. Zowel positieve als negatieve effecten zijn beschreven bij o.a. bilirubine, creatinine, ijzer, ureum, urinesuur, maar de meest frequent gerapporteerde parameter is fosfaat.

De Sia water test kan een eerste aanwijzing zijn voor deze interferentie, maar de bevestiging ervan is af te leiden uit de graad van het reactieverloop of bij analyse van een seriële verdunning van het monster, of is zichtbaar bij het manueel uitvoeren van de test. De oplossing bestaat erin de eiwitten in het staal te elimineren, de analyse uit te voeren met een andere methode of het monster serieel te verdunnen.

Goede communicatie tussen het laboratorium en de kliniek kan belangrijke klinische gevolgen tengevolge van analytische interferenties vermijden.

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