Two Cases with Unusual Vancomycin Measurements

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CASE

Patient A was a 68-year-old woman with a history of lymphoplasmaacytic lymphoma who presented after several weeks of mucosal bleeding. On admission, her cancer involved 95% of bone marrow cells. She was pancytopenic and febrile (38.3 °C). Leukocyte count was 0.39 × 10^9/μL (neutrophils, 0.03 × 10^9/μL), hematocrit 25%, platelet count 21 × 10^9/μL, relative serum viscosity 2.3 (reference interval, 1.4–1.8), blood urea nitrogen 6.4 mmol/L (18 mg/dL), creatinine 62 μmol/L (0.7 mg/dL), and estimated glomerular filtration rate >60 mL/min/1.73 m². She had an IgM monoclonal component of 42.8 g/L. This patient was started on vancomycin 1 g intravenously (IV) every 12 h, ceftazidime 2 g IV every 8 h, and a course of chemotherapy. On day 3 after the beginning of antibiotic treatment, a trough specimen was collected for measurement of vancomycin. The concentration, measured with a Beckman Coulter Synchron competitive turbidimetric immunoassay, was <0.1 mg/L. The result, which was incompatible with ongoing vancomycin therapy, signaled a problem to the technologist. No analytical issues were evident upon review of calibration, controls, and results for other chemistry tests performed on the same specimen. In an attempt to resolve an apparent falsely low result, a 1:1 mix of the specimen was made with the Beckman liquid comprehensive control serum (level 3, 30.4 mg/L) and demonstrated near complete recovery (result of mix after adjusting for dilution, 28.6 mg/L). However, a 1:1 mix with pooled patient serum containing vancomycin (11 mg/L) led to only 15% recovery (result of mix after adjustment for dilution, 1.7 mg/L). The specimen was subsequently sent to another laboratory, where a vancomycin concentration of 9.8 mg/L was measured by use of a competitive enzyme-linked immunoassay (Emit, Ortho-Clinical).

Patient B was a 64-year-old woman with a history of non-Hodgkin lymphoma admitted for stem cell transplantation. Her hospital course included acute renal failure, mental status changes, and disseminated intravascular coagulation. Leukocyte count was 2.18 × 10^9/μL (neutrophils 1.79 × 10^9/μL), hematocrit 28%, platelet count 7 × 10^9/μL, blood urea nitrogen 23 mmol/L (64 mg/dL), creatinine 160 mmol/L (1.8 mg/dL), and estimated glomerular filtration rate of 22 mL/min/1.73 m². She had an IgM monoclonal component of 10.0 g/L, with decreased normal gammaglobulins. This patient was started on vancomycin 750 mg IV every 24 h and imipenem-cilastatin 250 mg IV every 8 h for fever of unknown origin (38.2 °C). On day 2, a vancomycin trough specimen was collected just before administration of the second dose. The result was suppressed by the analyzer, which reported “reaction rate high,” i.e., faster than would be seen even in the absence of vancomycin. Calibration, controls, and the day’s vancomycin test results were reviewed and showed no problems. The inappropriate reaction rate repeated with dilution, and also occurred with analysis of 2 other specimens from patient B. The specimen was subsequently sent to another laboratory, where a vancomycin concentration of 6.9 mg/L was measured with a competitive enzyme-linked immunoassay (Emit, Ortho-Clinical).

DISCUSSION

WHAT MIGHT HAVE CAUSED THE PROBLEMS IN MEASURING VANCOMYCIN OBSERVED IN THESE TWO PATIENTS?

Vancomycin was initially measured with a particle-enhanced turbidimetric inhibition immunoassay. Free vancomycin present in the patient’s specimen competes with particle-bound vancomycin for drug-specific antibody binding sites, thereby inhibiting antibody-mediated particle aggregation. The rate and amount of particle aggregation are inversely proportional to the amount of vancomycin present in the patient’s specimen.

Both patients had high concentrations of monoclonal immunoglobulins, also called paraproteins, in their serum, which, as noted by the manufacturer, may potentially interfere. Paraproteins can promote aggregation via nonspecific mechanisms (aggregation not mediated by antivancomycin antibodies), which can lead to falsely low results, as seen for patient A, or error codes indicating aggregation rates that exceed those expected even in the absence of vancomycin, as seen for patient B.

When unexpectedly low drug concentrations or abnormally high reaction rates are detected, one can test for the presence of a nonspecific aggregator by mixing the specimen with an equal amount of a serum pool with a

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known vancomycin concentration. The measurement of a concentration that is substantially less than the mean of the 2 specimens suggests the presence of a nonspecific aggregator. After mixing the specimen from patient A with an equal volume of patient pool containing vancomycin, the apparent concentration was far less than expected. It is important to note that when a 1:1 mix was prepared using the manufacturer’s control (30.4 mg/L) instead of the patient pool, near complete recovery was observed. This result was most likely due to a matrix effect attributable to ethylene glycol in the Beckman control, which, we hypothesize, inhibits nonspecific aggregation, but does not affect antibody-mediated aggregation. Under less suspicious circumstances, the second mix might have been misinterpreted as confirmation of a subtherapeutic concentration, leaving the presence of a nonspecific aggregator undetected.

The specimens from both patients were subsequently measured by using assays that did not depend on particle aggregation and yielded concentrations that were not only consistent with the patients’ renal function and dosing regimens, but were also within the traditional target range of 5–10 mg/L for trough specimens. We also attempted to separate the interference from the patient samples by using ultrafiltration with a 100-kDa molecular weight cutoff filter. For both patient samples, filtration enabled detection of vancomycin in the patient samples and in 1:1 mixes, with the patient pool containing vancomycin (data not shown). This result provides strong evidence for a high–molecular-weight nonspecific aggregator in the patient samples, consistent with the IgM paraprotein in each sample.

WHAT OTHER LABORATORY TESTS MIGHT DEMONSTRATE INTERFERENCES FOR THESE PATIENTS?
Paraproteins can cause interference in other clinical assays through a few common mechanisms: production of turbidity in the specimen during the course of reaction or analysis, binding of the paraprotein to a component of the assay system, or binding of the paraprotein to the analyte itself. However, paraprotein interference is relatively uncommon. IgM proteins are the largest and most polyvalent immunoglobulins (molecular weight 900 kDa). As a result, IgM paraproteins cause most reported interferences with chemistry assays, although IgM gammopathies occur least frequently (1).

Paraprotein interference has been documented with glucose, bilirubin (both total and direct), HDL (2), γ-glutamyltransferase, urea, ferritin (1), and many other assays. The 2 incidents we describe appear to be the first reported cases of paraproteinemia interference with a vancomycin assay.

Most clinical assays are formulated to avoid interference by monoclonal immunoglobulins. In addition, most analyzers have software options that can detect anomalous reaction kinetics (e.g., the “reaction rate high” flag that occurred with the sample from patient B) (1). Specimens that do not follow normal reaction patterns can be identified, diluted, and retested. Dilution of patient specimens containing increased concentrations of aggregated paraproteins will often either completely or partially eliminate this type of interference (3). However, even with these protective mechanisms in place, such interferences may not be detected.

Less commonly, a paraprotein may bind the analyte being assayed or a component of the assay system. For example, if the paraprotein preferentially binds to any assay component (e.g., enzymes or antibodies that are part of an assay reaction scheme, or even the test analyte itself), falsely increased or decreased test results can occur (4). This type of interference will not usually be detected by anomalous reaction kinetics but rather when the unexpected result does not fit with the patient’s clinical picture.

Another potential source of assay interference with higher concentrations of IgM is increased specimen viscosity, particularly associated with Waldenström macroglobulinemia. On refrigeration these specimens can become gel-like, and inadequate warming may lead to pipetting errors (5). If the laboratory is aware of the patient’s condition, this temperature-dependent viscosity problem can be avoided.

WHAT ARE THE RECOMMENDATIONS FOR MONITORING VANCOMYCIN?
Vancomycin is a glycopeptide antibiotic that inhibits cell-wall synthesis and is primarily prescribed for patients with gram-positive infections. Vancomycin is not metabolized, but undergoes renal elimination with a clearance rate that approximates the glomerular filtration rate. As with other therapeutic drug monitoring, serum vancomycin measurements can be used to ensure effective dosing and to prevent toxic drug concentrations.

Despite decades of vancomycin use, there remains considerable debate regarding its toxicity and the necessity of measuring serum concentrations to prevent toxicity. This debate most likely stems from a paucity of studies that relate treatment outcome to serum concentrations in patients without confounding factors such as concurrent aminoglycoside antibiotic therapy. Current guidelines suggest that monitoring of serum concentrations is usually unnecessary in patients with normal renal function and uncomplicated infections being treated with standard doses of vancomycin. Monitoring of trough concentrations is suggested for patients receiving prolonged IV treatment, patients receiving other nephrotoxic drugs, patients who are mor-
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POINTS TO REMEMBER

- Paraproteins can promote nonspecific aggregation and thus interfere with particle-aggregation–based assays.
- Paraproteins occasionally interfere with other types of assays by increasing specimen viscosity, by causing turbidity, or by binding to an assay component or the analyte itself. These interferences may be resolved by sample dilution.
- IgM monoclonal immunoglobulins (e.g., those seen in Waldenström macroglobulinemia) cause disproportionately high degrees of interference.
- Current guidelines do not recommend routine monitoring of trough serum vancomycin concentrations.
- Therapeutic drug monitoring for vancomycin is recommended for complicated infections or when therapy includes prolonged intravenous administration or concurrent nephrotoxic drugs, and for patients who are morbidly obese, have an altered volume of distribution, or have unpredictable/impaired renal function.

bidly obese, patients with an altered volume of distribution, and patients with unpredictable or impaired renal function (6). The traditional target range for trough concentrations is 5–10 mg/L, but recent guidelines have suggested a target trough range of 15–20 mg/L for complicated and hospital-acquired infections (7). There is currently a general consensus that monitoring of peak concentrations is of little benefit (6, 8).

In the cases we report, because patient A had normal renal function, monitoring vancomycin was probably not indicated. This conclusion is supported by the finding of a trough concentration within the traditional target range.

PATIENT FOLLOW-UP

The team caring for patient A opted to wait for results from the outside laboratory rather than make immedi-

ate changes to the dosing regimen. Because renal function was normal, no additional testing was requested once the normal results from the outside laboratory were received. In contrast, the team caring for patient B discontinued the drug before the next dose owing to her tenuous renal status. Each case highlights the issues that may be seen with monoclonal gammapathies and the importance of policies to prevent the inappropriate release of inaccurate results.

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References


Commentary

David F. Keren

These cases remind us that IgM monoclonal proteins (M-proteins) have earned a reputation as saboteurs of many laboratory assays owing to their self-aggregation, aggregation of latex particles, binding to analytes and reagents, and cryoprecipitation. One additional point is that some IgM M-proteins also play havoc with their own detection and measurement by electrophoretic techniques.

Immunoelectrophoresis, an older technique still used by a dozen or so laboratories, employs a long gel-diffusion step during which large IgM molecules move through agarose more slowly than the sleeker IgG, result-

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ing in a phenomenon known as the umbrella effect. This effect results in false negatives when the IgM M-protein light chain is obscured by the polyclonal light chains of IgG (1). The solution employed for this problem is to break the disulfide bonds with 2-mercaptoethanol. Immunoelectrophoresis has been largely replaced by immunofixation. One of the many advantages of immunofixation is that the reagent antisera are placed directly on the gel, so a long diffusion phase is not required and the umbrella effect is virtually eliminated. Another problem with IgM measurement has emerged recently with capillary zone electrophoresis. With this technique, IgM M-protein measurement occasionally yields markedly lower quantities than those obtained with nephelometry or densitometric scan of serum protein electrophoresis on agarose gels (2). As with the umbrella effect in immunoelectrophoresis, capillary zone electrophoresis measurement of the IgM M-protein is improved by pretreatment with 2-mercaptoethanol, suggesting that factors relating to size or self-aggregation may interfere with the passage of M-proteins through the narrow capillary. In my laboratory we recommend that the first time an IgM M-protein is identified by capillary zone electrophoresis, it should be subjected to agarose gel electrophoresis to compare the quantity of the M-protein. If there is a disparity, we recommend following the M-protein by densitometric scans using agarose gel electrophoresis or by nephelometry for measuring total IgM concentration. There remains an important role for keen scrutiny of data by laboratorians to prevent spurious results from affecting clinical decisions.

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References

globulins are high–molecular weight molecules, re-
analysis of this specimen by use of protein-free ultrafil-
trate (free vancomycin) may have eliminated this
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**Reference**

1. Biedenbach DJ, Bell JM, Sader HS, Fritsche TR, Jones RN, Turnidge JD.
Antimicrobial susceptibility of Gram-positive bacterial isolates from the
Asia-Pacific region and an in vitro evaluation of the bacteriocidal activity
of daptomycin, vancomycin, and teicoplanin: a SENTRY Program Report