Q&A column, 2/17

Editor: Frederick L. Kiechle, MD, PhD

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Submit a Question

Q. I have an oncology patient with a diagnosis of immune thrombocytopenia. The patient's sample has been drawn in sodium citrate, EDTA K2, sodium heparin, and warm saline replacements, and a true platelet count cannot be obtained. Platelets clump in all tubes, and multiple platelet clumps are observed under the microscope. The patient doesn't have thrombocytopenia. What else can I do?

A. Pseudothrombocytopenia—known more colloquially as platelet clumping—is a relatively rare phenomenon but one of great frustration to the hematology laboratory. In most instances, pseudothrombocytopenia results from an in vitro EDTA-dependent antiplatelet antibody activation, resulting in platelet aggregation.[1] These antiplatelet antibodies may arise in a variety of clinical scenarios including autoimmune disorders, after trauma or surgery, with infection, as a drug effect, or in the context of a variety of malignancies.[1] The nature of these autoantibodies can vary, including IgM, IgG, and IgA subtypes and a strong association with concurrent antiphospholipid antibodies.1 Fortunately, unlike other platelet disorders (e.g. heparin-induced thrombocytopenia) relating to antiplatelet antibodies, pseudothrombocytopenia is not typically associated with in vitro platelet activation in and of itself.

Despite the name "pseudothrombocytopenia," an underlying true thrombocytopenia may or may not be present. To overcome this in vitro bias, a variety of procedural remedies have been suggested. The use of heparinized tubes, rather than EDTA collection tubes, has shown some success.[2] Other authors have also suggested that the addition of an aminoglycoside antibiotic may be useful.[3] A variety of other remedies (including the use of antiplatelet agents such as aspirin) are described in an editorial by Lippi and Plebani.[1]

Our laboratory recently encountered a fairly stubborn case, in which none of the methods using heparin or warm saline were effective at overcoming pseudothrombocytopenia. Our flow cytometry laboratory was enlisted, not only to provide confirmation of antiplatelet antibodies present but also to provide an estimate of platelet count by orthogonal means. Subsequent blood sampling was also altered to incorporate microtainers (see, for example, the BD Tech Talk article from 20104); these were enlisted to try to minimize the physical forces applied to sample blood, as well as to optimize blood-anticoagulant mixing within the specimen containers. Care was also taken to ensure that containers were pre-warmed and maintained at physiologic temperatures. If these remedies continue to result in automated analyzer "fails," the use of microscopic hemocytometers (by which clumped platelets may be enumerated in a known standard volume) can also be attempted, to at least provide an estimate of the platelet count.

1. Lippi G, Plebani M. EDTA-dependent pseudothrombocytopenia: further insights and recommendations for prevention of a clinically threatening artifact. *Clin Chem Lab Med.* 2012;50(8):1281–1285.
2. Chae H, Kim M, Lim J, Oh EJ, Kim Y, Han K. Novel method to dissociate platelet clumps in EDTA-dependent pseudothrombocytopenia based on the pathophysiological mechanism. *Clin Chem Lab Med.*

2012;50(8):1387–1391.
3. Ozcelik F, Arslan E, Serdar MA, et al. A useful method for the detection of ethylenediaminetetraacetic acid- and cold agglutinin-dependent pseudothrombocytopenia. *Am J Med Sci.* 2012;344(5):357–362.
4. Leslie M. Why do platelets sometimes clump in EDTA tubes? Tech Talk. BD Global Technical Services. 2010;8(3).

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Q. The presence of albuminuria may be the first indicator of early renal disease. Urine albumin is determined primarily by an immunological method; some authors have said, however, that this method excludes a large component of non-immunological albumin. If this is true, won't there be a marked decrease in the sensitivity of the "true" urine albumin level, delaying the detection of already existing renal disease and thus potentially delaying needed therapy?

A. I believe the question refers to the claim that Wayne Comper, PhD, DSc, and collaborators made more than a decade ago that a substantial fraction of the albumin found in urine is immunologically altered or fragmented in a way that makes it undetectable by typical immunologically based urinary albumin measurement procedures.[1] Dr. Comper and colleagues claimed that use of size-exclusion high-performance liquid chromatography (HPLC) was a superior methodological approach for measuring urinary albumin and it allowed for earlier detection of patients at higher risk of developing chronic kidney disease than do immunologically based urinary albumin measurement procedures, particularly in diabetics.[2]

The assertion that HPLC-based urinary albumin measurement procedures are diagnostically superior to immunologically based ones for early detection of renal function decline and premature mortality has been controversial, with dozens of published research reports supporting one side or the other. One of the earlier reports from the NIH Clinical Center suggests that size-exclusion HPLC lacks analytical selectivity since proteins other than albumin are being measured by this method.[3] The accompanying editorial also recommends caution in adopting the size-exclusion HPLC methodology.[4] Virtually all published evaluations of the HPLC urinary albumin measurement procedure show that it gives higher urinary albumin results, often severalfold higher, than immunologically based measurement procedures, particularly in the very mildly elevated range (e.g. urinary albumin to creatinine ratios of 20 to 100 mg/g creatinine). However, several studies looking at clinical endpoints of renal function decline and mortality suggest that, when looking at the entire range of albumin excretion per 24 hours or albumin creatinine ratio using receiver operating characteristic curves, little advantage is seen for the HPLC approach versus immunological measurement procedures in separating patients who are destined to experience renal function decline or early mortality.[5]

Several fairly comprehensive reviews of how best to measure urinary albumin and interpret those measurements have been produced by the International Federation of Clinical Chemistry and Laboratory Medicine and the National Kidney Disease Education Program Laboratory Working Group on standardization of albumin in urine.[5,6] One of these reviews, in *Critical Reviews in Clinical Laboratory Sciences*,[6] attempts to address the reader's question fairly specifically about the relative merits and interpretation of the results from HPLC versus immunologically based measurement procedures. The authors' conclusion is that "The existence of immunochemically unreactive albumin in urine has been questioned and until now the detection of this albumin

form provides no advantage to standard approved urinary albumin assays," and they recommend the use of immunoassays with polyclonal antisera since they react with many modified albumin forms.

Both of these comprehensive reviews of the literature on urinary albumin measurement emphasize a need for better standardization and development of a more robust reference system for immunological albumin measurement procedures so that the current immunologically based clinical laboratory methods yield more comparable results. The need for better standardization of urinary albumin results among clinical laboratories is further emphasized in a 2014 article reporting that some currently available commercial immunological urinary albumin measurement procedures can produce results with biases as large as 35 percent compared with isotope dilution mass spectrometry reference measurement procedures.[7]

In summary, the consensus seems to be that size-exclusion HPLC urinary albumin measurements offer little clinical diagnostic advantage but that there is an urgent need for improvement in accuracy of many of the commercially available immunologically based clinical measurement procedures if clinicians are to be given reliable urinary albumin results for treating patients.

1. Comper WD, Osicka TM, Jerums G. High prevalence of immuno-unreactive intact albumin in urine of diabetic patients. *Am J Kidney Dis.* 2003;41[2]:336–342.
2. Osicka TM, Comper WD. Characterization of immunochemically nonreactive urinary albumin. *Clin Chem.* 2004;50[12]:2286–2291.
3. Sviridov D, Meilinger B, Drake SK, Hoehn GT, Hortin GL. Coelution of other proteins with albumin during size-exclusion HPLC: implications for analysis of urinary albumin. *Clin Chem.* 2006;52[3]:389–397.
4. Peters T Jr. How should we measure the albumin in urine [editorial]? *Clin Chem.* 2006;52[4]:555–556.
5. Miller WG, Bruns DE, Hortin GL, et al.; National Kidney Disease Education Program–IFCC Working Group on Standardization of Albumin in Urine. Current issues in measurement and reporting of urinary albumin excretion. *Clin Chem.* 2009;55[1]:24–38.
6. Speeckaert MM, Speeckaert R, Van De Voorde L, Delanghe JR. Immunochemically unreactive albumin in urine: fiction or reality? *Crit Rev Clin Lab Sci.* 2011;48[2]:87–96.
7. Bachmann LM, Nilsson G, Bruns DE, et al. State of the art for measurement of urine albumin: comparison of routine measurement procedures to isotope dilution tandem mass spectrometry. *Clin Chem.* 2014;60[3]:471–480.

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