Biotin interference: answering questions, reducing the risk

David Wild

November 2018—Biotin use is not rare, and don't count on it being listed in the patient's electronic medical record. Those are some of the findings of a Mayo Clinic study published recently in *Clinical Biochemistry* (Katzman BM, et al. 2018;60:11–16).

Mayo Clinic's laboratory isn't alone in having fielded questions from clinicians about biotin interference in immunoassays. Nikola Baumann, PhD, DABCC, co-director of Mayo Clinic's central clinical laboratory and central processing, who spoke at this year's AACC annual meeting on what Mayo is doing to limit the interference risk, said her laboratory's research and experience, and that of others, has provided sufficient data to help in answering some of the most common questions, though the answers "aren't black and white."

Two such questions: How prevalent is biotin use, and who is taking it? In one week in July 2017, she and colleagues surveyed 4,000 Mayo Clinic outpatients who presented for scheduled blood collections. Of those surveyed, 1,944 returned completed paper questionnaires (972 female, 963 male, nine unspecified).

"I found it quite surprising that eight percent said, yes, they were taking biotin," Dr. Baumann said in her presentation. "And what was also interesting is that five percent didn't know if they were taking biotin. So people are taking over-the-counter supplements and may not even be aware what they're taking." Of those who said they consumed biotin, 79 percent were female, 21 percent male. Median age was 62.

In the same study, biotin was quantified in 1,442 residual waste plasma samples collected for physician-ordered electrolyte panels from patients presenting to the emergency department in March 2017. Nearly 50 percent had detectable biotin concentrations. Dr. Baumann and coauthors wrote, "There was an alarmingly high percentage of samples (7.4%) with biotin concentrations that fell at or above the lowest thresholds for assay interference (10 ng/mL) reported by Roche Diagnostics." Nearly two percent of samples had biotin concentrations greater than or equal to 20 ng/mL.

Dr. Baumann

In the 107 samples in which biotin measured greater than or equal to 10 ng/mL, the EMR was reviewed to determine if biotin or multivitamin use had been noted. For only two was biotin use noted in the EMR. Thirty-three patients had multivitamin use listed. "One of the interventions that has been proposed is to use clinical decision support tools to query medication lists, but in our experience we have found that would not be robust," Dr. Baumann said, because biotin use is often not listed in the EMR.

During the two-week period in which samples were obtained, only one patient in the study cohort had a physician-ordered test that would have been affected by his plasma biotin concentration. The authors say this may be a limitation of the study as residual samples were obtained from patients with a physician-ordered electrolyte panel rather than their targeting patients on whom immunoassays were ordered. "However, even with this conservative estimate," they write, "we could extrapolate that 26 patients in the ED per year would be expected to have an erroneous laboratory result due to biotin interference."

Another common question for laboratories: What serum biotin concentrations can laboratories and providers expect to see in patients taking biotin supplements in the range of 1,000 to 10,000 mcg/day?

In 2015, when Mayo Clinic's laboratory began to investigate the interference problem, "we really didn't have much information to go off of," Dr. Baumann said, other than manufacturer package inserts.

One manufacturer suggests blood not be drawn from patients taking high biotin doses until at least eight hours after the last biotin dose. The biotin threshold provided is less than 25 ng/mL. Another manufacturer indicates that laboratories should expect to see a 7.3 percent decrease in T_3 results at 10 ng/mL. "So manufacturer package inserts take different approaches," Dr. Baumann noted.

Then, in 2017, a study conducted by Roche Diagnostics and published in the *International Journal of Pharmacokinetics* examined serum concentrations in healthy subjects taking 5-, 10-, or 20-mg biotin daily (Grimsey P, et al. 2017;2[4]:247–256).

In the study, serum concentrations when blood samples were drawn one hour post-dose were 40.5 ng/mL among those administered 5 mg/day, 90.6 ng/mL among those administered 10 mg/day, and 183.8 ng/mL among those taking 20 mg/day. Three hours after a biotin dose, serum concentrations dropped to 16.2 ng/mL in the 5-mg group, 32.7 ng/mL in the 10-mg group, and 63.7 ng/mL in the 20-mg group. Twelve hours after a dose, biotin levels were 4.9 ng/mL in the 5-mg group, 10.3 ng/mL in the 10-mg group, and 20.9 ng/mL in the 20-mg group.

After five days of biotin supplementation, peak serum concentrations were comparable to those of day one, with levels decreasing by 12 hours but not returning to baseline, a pattern that points to "a bit of a cumulative effect that needs to be investigated further," Dr. Baumann said.

"What's interesting here is that biotin is rapidly absorbed and there is a broad distribution of concentrations following a single dose of biotin," she said.

Dr. Baumann uses a total hCG assay in use at Mayo to put the results in context. It has a biotin threshold in the package insert of 80 ng/mL. "In the case of this assay, most of the patients would be below this threshold by about three hours post-dose."

With a free T_4 assay used in her laboratory, which has a manufacturer threshold of 20 ng/mL, the serum concentrations of patients on 20-mg biotin consistently over several days could be above the threshold even 12 hours post-dose. "So, when I'm asked the question, 'how long should a patient refrain from biotin?' the answer is, 'it depends,'" Dr. Baumann said.

At Mayo Clinic, patients are instructed to refrain from biotin supplements for at least 12 hours to make it consistent with fasting instructions. "However, the endocrinologists themselves ask patients whether they take biotin, and if the patients have been taking biotin for a long time, the endocrinologists ask them to refrain from biotin use for a week."

While the Roche pharmacokinetic study provides important insights, it does not capture some of the "complexities" of biotin use, Dr. Baumann said. For example, while study subjects were healthy, patients with impaired renal function excrete biotin more slowly, and Mayo has had a few cases in which interference was still being observed more than 72 hours after a biotin supplement was taken. "So these are hard questions to answer, but I think these data give us an idea that if you're taking a regular low-dose biotin, refraining from biotin use for 12 hours before a sample is drawn is probably enough." If patients are taking higher-dose biotin for long periods, they need to refrain for a longer period. "And it also depends on the threshold of the assay."

How biotin is used in immunoassays is another common question, she said. The vitamin can be part of the soluble components of the reagent—free biotinylated analogs and antibodies and streptavidin-coated particles—or part of pre-complexed reagents, where the biotin streptavidin complexes are preformed in the reagent. "Or biotin can be not used at all in the assay design, in which case the manufacturer is usually using anti-animal antibodies instead."

Dr. Baumann and her colleagues conducted experiments spiking biotin in vitro into two assays used in their lab, and measured samples in triplicate using Roche's Cobas e 602 immunoassay module. For a competitive T_3 immunoassay, "we used a 10 percent bias as what we considered to be a clinically significant interference." They found a "fairly linear response" and a threshold of about 19.3 ng/mL. For a TSH sandwich immunoassay, they found decreasing TSH with increasing biotin concentrations.

Mayo Clinic's quest to understand and mitigate the impact of biotin interference began in July 2015, when a physician questioned a patient's free T_4 lab results. In September of the same year, biotin interference was confirmed as the reason for the discrepant results on that patient, and a month later Dr. Baumann and colleagues began retesting all samples with elevated free T_4 to check for interference.

"That was the safety net we thought would be the easiest to implement," Dr. Baumann said. "We also communicated with the clinical practice that had found this case. We wanted to raise awareness."

Using the elevated free T_4 retesting protocol and an assay that didn't have biotin interference, the laboratory in December 2015 identified three additional cases of interference. Those results led them to add instructions to their patient appointment guides. "In addition to fasting instructions, we now have a recommendation to refrain from biotin supplements for 12 hours as well as an explanation of where patients might find biotin," Dr. Baumann said.

In January 2016, they developed a streptavidin-based protocol to confirm biotin interference, in what Dr. Baumann calls "an ongoing journey for the past couple of years."

"Our current state is that we continue to retest samples with elevated free T_4 results," she said, adding that they have "loosened the safety net a little" and now retest only the samples that contain more than 2.5 ng/dL. "Our clinician awareness now is very high, and our endocrinologists absolutely know about biotin and they're discussing it with their patients, but we still do find a case every once in a while."

Mayo Clinic tests roughly 2,900 blood samples for free T_4 every month, and if a result is less than or equal to the upper end of their reference interval of 1.7 ng/dL, they report those results to the clinician. "A limitation of our retesting strategy and of our safety net is that a patient could have low free T_4 but it would show up as normal because of biotin interference, and we would not have caught those samples," Dr. Baumann said.

About 10 percent of all free T_4 results at Mayo Clinic fall above the reference interval, and in those cases, they retest using an alternative platform not affected by biotin.

"If those results are comparable, which we define as a difference of less than 30 percent because we know there's about a 20 percent difference in those free T_4 assays, we consider there to be no interference and we report the results to clinicians," Dr. Baumann explained. "If we find a difference of greater than 30 percent between those two results, we'll perform our biotin interference workup, which includes streptavidin depletion studies."

In using this retesting protocol for two years, Mayo Clinic has found 17 confirmed cases of biotin interference. "What I think is striking is that 15 of those cases were found after we had implemented patient instructions that explicitly say to refrain from using biotin" before a draw, Dr. Baumann said.

That finding can be interpreted in two ways, she said. One is that the patient instructions are effective and 15 cases is a low number. "We don't know if without patient instructions we would have found 50, 60, 100 cases." Another interpretation: Patients are not reading their instructions and perhaps the 15 cases are a baseline, something she called "very possible" and "disheartening."

It's difficult to know which way to understand the data, Dr. Baumann said, because "we don't know what our pre-patient-instruction interference frequency was." Despite that, it's her view that "one of the best interventions is to raise awareness on both the clinician side and patient side."

Illustrating the impact of the laboratory's retesting strategy, Dr. Baumann recalled one case in which an initial test

found 3.8 ng/dL free T_4 with a TSH of 0.05 mIU/L. Those levels prompted retesting using an alternative platform not affected by biotin and which found a free T_4 level of 1.1 ng/dL and a TSH of 3.5 mIU/L, both within the normal range.

“We treated that sample with streptavidin, did our streptavidin agarose depletion experiment, and found that if we now measured it using our primary method, the free T_4 was 1.2 and the TSH was 3.4, which compared very well with our alternate method,” Dr. Baumann said.

Pointing to 22 confirmed cases of biotin interference detected by the retesting protocol or by a physician questioning the pattern of results, Dr. Baumann said biotin causes “a perfect storm” when it comes to thyroid function testing. “It causes a perfect scenario of hypothyroidism. I would say the one red flag is that often TSH is not completely suppressed like you would expect it to be in the context of the really high free T_4 levels. That is your one warning sign.”

As Mayo Clinic’s experience indicates, biotin interference can be clinically relevant: Of the 22 cases in which biotin interference affected free T_4 levels, 16 patients had high free T_4 with low TSH while six had high free T_4 and TSH within the reference interval. “So that might cause someone to raise an eyebrow to call the lab to ask a question.”

After retesting the 22 samples with methods that don’t have biotin interference, 15 samples were found to have normal free T_4 and TSH levels, three patients had high free T_4 and low TSH (clinically consistent with hyperthyroidism), and four had normal free T_4 levels with high TSH levels based on the reference interval. “But in all of those cases the TSH was still less than 6.7, which is not really considered elevated,” she noted.

In contrast to 2015, when little was known about the nature and impact of biotin interference, Dr. Baumann and her colleagues now have enough knowledge and experience to answer this question from clinicians: Is there a risk of misdiagnosis with biotin interference? “The answer is yes,” she said. “It’s something that labs, clinicians, and patients need to be aware of.”

David Wild is a writer in Toronto. The session in which Dr. Baumann spoke was a joint AACC-Endocrine Society symposium.