No perfect approach to detecting *C. diff* infection

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May 2017—With *Clostridium difficile* causing a wide range of infectious manifestations, the dilemma for clinical laboratories is how to balance the different diagnostic options. “Because if you’re treating someone who is only colonized, you’re not going to benefit them—and very likely you may harm them,” said Ferric C. Fang, MD, professor of laboratory medicine, Department of Microbiology and Medicine, University of Washington School of Medicine, Seattle, in a recent webinar hosted by CAP TODAY and sponsored by BioFire. And having a negative toxin assay is no assurance, he said, that *C. difficile* is not causing disease.

Dr. Fang

In the webinar on *C. diff* in the community setting (Erik R. Dubberke, MD, MSPH, co-presented, see page 46), Dr. Fang reviewed the diagnostic assay options for *C. diff.* Among them are tissue culture, which he called “cumbersome” and “not completely sensitive clinically”; a toxigenic culture, which he called “too slow to be very useful clinically”; toxin EIA, an immunoassay that detects one or both of the toxins made by C. diff; and multistep algorithms, which use a screening test.

“Glutamate dehydrogenase protein can be used to detect *Clostridium difficile*, but it won’t distinguish between toxigenic and nontoxigenic strains,” he said. “This has to be followed up by a toxin assay or by a PCR assay for the presence of the toxin gene.”

The newest approach, molecular detection for cytotoxic *C. diff*, is highly sensitive but expensive, while GDH and toxin EIAs are convenient, relatively inexpensive, and popular.

The nucleic acid amplification tests come in a variety of platforms, he noted. “They can be performed as standalone tests, or they can be present as part of an enteric panel. Because they are highly sensitive for the presence of toxigenic *C. difficile*, if you look at the spectrum of illness, I would agree that they’re more likely to detect colonized patients than a less sensitive assay like an immunoassay. And they may be more likely also to detect patients with milder illness. But on the other hand, because they’re more sensitive, you’re going to have less of a problem of missing significant cases.”

Regarding the screening GDH assay, he pointed to a multicenter blinded trial showing a strain specificity for the detection of GDH (Tenover FC, et al. J *Clin Microbiol.* 2010;48[10]:3719–3724).

“For the 027 highly virulent outbreak-associated strain, the GDH algorithms were actually just as sensitive as PCR,” he said. “This is not because there’s a difference in GDH expression by different strains, but probably because the 027 strain is more virulent and tends to have higher organism burden,” which is easier to detect with an immunoassay. “But for the other ribotypes of *C. difficile*, there was a substantial loss in sensitivity. It’s 15 to 20 percent less sensitive. These are *C. difficile*-positive stools that would be missed by using a GDH screen.”

To illustrate, he recalls a 63-year- old woman referred from another hospital to UW with a diagnosis of suspected inflammatory bowel disease. She had had a bout of diverticular disease and been treated at the other hospital with antibiotics, then began experiencing diarrhea, weight loss, occasional fecal incontinence, and bloody stool. After

oral antibiotics didn't help, C. diff was suspected.

"So they sent stools for immunoassay, and they sent repeated specimens knowing there was an issue with sensitivity, but they were all negative," Dr. Fang said. "Then she was referred to us for evaluation by the GI service and a colonoscopy, and we performed a GDH screen, which was part of our algorithm at the time, and this was also negative. But the patient underwent colonoscopy and she had colitis, but not pseudo membranes. So no pathologic findings that were pathognomonic for *C. difficile*." The working diagnosis made by the chief of the GI service was inflammatory bowel disease. And the physician was preparing to initiate a course of immunosuppressive therapy.

"But at the time, we were evaluating a PCR assay," he continued, "and we were surprised to find that this GDH-negative stool was PCR positive for C. difficile. Culture of the organism showed it was toxigenic. So the clinician decided instead of immunosuppressing the patient to try oral vancomycin first, which had never been tried. And the patient had complete resolution of her symptoms with a 10-day course of vancomycin, and she did not recur. This is a case that shows potentially catastrophic consequences if the patient had been placed on immunosuppressive therapy for what turned out to be *C. difficile* infection because the laboratory had missed the diagnosis relying on a GDH screen."

Some laboratories, he noted, use GDH screening followed by a toxin immunoassay. Some follow GDH screening and toxin immunoassay with PCR, in an attempt not to miss some toxin-negative specimens. Others perform PCR up front but then corroborate with a toxin immunoassay and consider the PCR-positive, toxin-negative specimens as indicative of colonization. And still others, such as Dr. Fang's own laboratory, use PCR alone.

"None of these approaches is perfect," he said. "Relying on GDH and toxin immunoassays is clearly insensitive. Backing up with PCR improves the sensitivity, but it creates a potential for overdiagnosis because of the PCR assay, and also [it creates] complex reporting because now you have three different assays you're applying, and recording this in the record can be confusing to clinicians. Also, the sensitivity is not perfect because the GDH immunoassay will miss some positive specimens. The PCR followed by toxin immunoassay can result in underdiagnosis because you're interpreting a negative toxin assay, positive PCR as colonization," which does not appear to be supported by the data, he said. "Finally, the reliance on PCR alone does have the potential for overdiagnosis if you're using the test in a clinically inappropriate setting."

One way to guard against inappropriate testing in the outpatient setting is to apply criteria from the American College of Gastroenterology, which recommends limiting testing for GI illness in looking for a diagnosis to patients who have moderate to severe illness, frank dysentery, symptoms that last longer than a week, or risk factors for severe illness and transmission, such as immunocompromised status (Riddle MS, et al. *Am J Gastroenterol.* 2016;111[5]:602–622). Using these criteria helps eliminate many cases of self-limiting outpatient diarrhea.

Dr. Fang reviewed a handful of studies on the role of PCR and other assays for *C. diff.* One study performed in the United Kingdom compared 435 patients who had positive cytotoxin assays with 207 patients who had positive toxigenic culture ("basically equivalent to a molecular assay") but negative cytotoxin assays, he said. The toxin-positive group had more deaths than the culture-positive, toxin-negative group. The researchers' conclusion, with which Dr. Fang did not agree: In the patients with a positive culture but negative toxin assay, C. diff infection was probably not the cause of diarrhea (Planche TD, et al. *Lancet Infect Dis.* 2013;13[11]:936–945).

He pointed out a couple of caveats about the study. First, "a significant percentage of the culture-positive, toxin-negative patients did, in fact, get empirical antibiotics for *C. difficile,* so it's difficult to evaluate the outcomes in this group as being indicative of the course of infection left untreated," he said. Second, "the culture-positive, toxin-negative patients had a significantly longer length of hospital stay compared to controls that were negative for both tests, suggesting that even though the mortality was not significant, the morbidity may have been significant, and the patients had longer stays in the hospital, which would also increase cost. Also, important endpoints such as persistent diarrhea were not measured."

A UC Davis study compared 131 patients who had a positive toxin EIA with 162 patients who had a positive PCR

assay/negative toxin EIA. “In this study, clinicians were not made aware of the PCR result,” he explained. The researchers found that serious complications were seen only in the group with the positive toxin. “The mortality was higher in the toxin-positive group than in the group that was PCR-positive only, and they had more prolonged diarrhea. So the two groups were different clinically. And they concluded that the molecular assay could result in overdiagnosis of *C. difficile* and over treatment” (Polage CR, et al. *JAMA Intern Med.* 2015;175[11]:1792–1801).

That study, too, had caveats, Dr. Fang noted: A significant percentage—about a fifth of the PCR-positive, toxin-negative group—when retested had converted to toxin-positive. “So being toxin-negative is not necessarily a stable state. And if you assume those patients are only colonized, they may subsequently go on to develop serious disease. And actually, a group at Johns Hopkins has shown that the relative risk of patients in the ICU who are toxin-negative, PCR-positive of subsequently developing symptomatic CDI is quite substantial, 10- to 15-fold above patients who are not colonized.”

In addition, at least 40 percent of these patients who were PCR-positive, toxin-negative received empirical antibiotics. “And the PCR-positive, toxin-negative group was more likely to be discharged with persistent diarrhea than the controls who had both tests negative. But looking at the clinical status of the patients, one concludes that even though the group with toxin positivity was sicker, the group with PCR-positivity, toxin-negativity had significant symptomatology.”

To any listeners who might have thought that the toxin assay or even a quantitative toxin assay could distinguish patients who have diarrhea from those with no significant diarrhea, Dr. Fang pointed to a Stanford study that found “essentially no difference in terms of the distribution of toxin concentrations in the stool of either toxin A or toxin B among patients who had significant diarrhea and no significant diarrhea” (Anikst VE, et al. *Diagn Microbiol Infect Dis.* 2016;84[4]:343–346). “This is based on clinical assessment. And this kind of experience certainly doesn’t give me any confidence that we can use the presence or absence of toxin to decide whether a patient is likely to have CDI or not.”

Meanwhile, other studies, which collectively included data from more than 2,000 patients, compared EIA positivity with patients who had either a PCR or a toxigenic culture positive only. Those studies found, Dr. Fang said, that the EIA was not predictive of *C. difficile* severity, mortality, recurrence, transmissibility, or pseudomembranous colitis. “And so many, many studies are failing to corroborate what other people are reporting,” he said. “This suggests that local differences may be necessary to consider in terms of the significance of having a positive PCR assay and a negative toxin assay. But taken together, you can see that just about exactly half of the significant cases of CDI would be missed by reliance on a toxin immunoassay. And for me, this is really an unacceptably high rate of false negativity.”

“It’s well documented in the literature that you can have very significant CDI, even life-threatening, in the absence of a positive cytotoxin assay,” he said. For example, a University of Pittsburgh study showed that having a negative toxin assay was, in fact, a risk factor for having fulminant *C. difficile*—a condition with 70 percent mortality. The patients found at autopsy to have C. diff infection were twice as likely to have a false-negative toxin assay (Dallal RM, et al. *Ann Surg.* 2002;235[3]:363–372).

Returning to the community setting, he pointed to a study from Madrid that found that community-acquired CDI is underdiagnosed. “In this study, they decided to perform a toxigenic culture on all diarrheal stools submitted to the lab, whether or not it was requested by the clinician,” he said. “And they found that more than 10 percent of the CDI was missed because the clinicians didn’t suspect it. And the risk factors for this were that the patients were younger, they were less likely to be on prior antibiotics, so they didn’t have the obvious risk factor. They were mostly community-acquired. And in the end, this result ended up being actionable in many cases” (Reigadas E, et al. *J Infect.* 2015;70[3]:264–272).

“So we have multiplex GI panels that have become very popular because they can replace the laborious stool workup,” he said. Multiple platforms are available, but they don’t all have *C. difficile.* “And the question before us is

whether it's appropriate to include *C. difficile* in these panels in the community setting used for the diagnosis of acute gastroenteritis." One way to look at this is to ask how often *C. diff* is found among the other pathogens on these panels, he added.

Several studies have shown that C. difficile is the second most prevalent target detected after enteropathogenic E. coli. But what about significance? Dr. Fang cited data from the Netherlands showing that in young children, "you're just as likely or more likely to find C. difficile that's toxigenic by PCR in asymp-tomatic children as you are in those with diarrhea. But if you look at all of the groups who are older than five years of age, you can see substantially more C. difficile detection by PCR in those who have diarrhea compared with asymptomatic controls. In fact, the detection in this study in older patients was virtually diagnostic for diarrhea, although other studies have shown a significant carriage rate depending on who you sample. So in the outpatient setting, it does appear that C. difficile detection by PCR correlates with the presence of symptoms" (Bruijnesteijn van Coppenraet LE, et al. *Clin Microbiol Infect.* 2015;21[6]:592.e9–592.e19).

One of the reasons for syndromic panels, Dr. Fang said, is that there is overlap between different etiologies of diarrhea. Clinicians who order *C. difficile* only may miss other pathogens; conversely, *C. difficile* may be present even if not tested for. "Clinicians have some ability to predict what they're going to find, but it's not perfect. And by having the multiplex panel and having this target on it, it can bring their attention to the possibility of *C. difficile*, which they might not have considered because of the community setting and the lack of traditional risk factors."

He summed up by saying: "My conclusion is that it's appropriate to include *C. difficile* on a multiplex GI panel provided that testing is performed only on patients who have significant or sustained symptoms per the clinical guidelines."
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Anne Ford is a writer in Evanston, Ill. The webinar can be viewed in full at here.