

And the band neutrophil counts play on

Karen Titus

December 2023—The recent CAP proficiency testing questionnaire was meant to be the coup de grâce. Hematology PT participants were asked about their band neutrophil reporting practices and, given that these manually generated counts were supposedly on their way out decades ago, the authors of the survey questionnaire expected to see very little activity. The survey, they hoped, would be a way to pound the final, data-driven nail in the coffin.

Or, as lead author Maria (Ria) Vergara-Lluri, MD, puts it, “We thought this had all been laid to rest 30 years ago.”

It wasn’t. Says Dr. Vergara-Lluri: “Surprise: 86 percent of labs that participated still report bands.”

The results of the survey (Vergara-Lluri M, et al. *Arch Pathol Lab Med*. Published online Aug. 28, 2023. [doi: 10.5858/arpa.2023-0015-CP](https://doi.org/10.5858/arpa.2023-0015-CP)) upended many of the assumptions, if not hopes, the authors might have had.

Among laboratories that reported manual differentials, they found that most reported bands (4,554 of 5,268). Moreover, only 73 percent reported band reference ranges.

On the morphologic challenge, bands classified as “easy” were indeed easy—participants classified them well (78 to 98.3 percent of respondents). But categorization of cell identifications for “moderate” and “difficult” bands was poor (3.1 to 39 percent of respondents), with classification instead as segmented neutrophils.

Moreover, there were no winners. Whether the laboratory was small, medium, or large, community based or academic, they all did poorly.

Now, instead of using the data to nail the lid shut, the authors will have to persuade physicians to first build a casket and put the body in it.

The authors, all of whom are current or former members of the CAP Hematology/Clinical Microscopy Committee, were spurred to do the survey in part because of band counting-related inquiries that coauthor Parul Bhargava, MD, found herself answering in CAP TODAY’s “Q&A” column.

“This was really Parul’s brainchild,” says Dr. Vergara-Lluri, associate professor of clinical pathology and attending hematopathologist, Keck School of Medicine of USC (University of Southern California) and Los Angeles General Medical Center.

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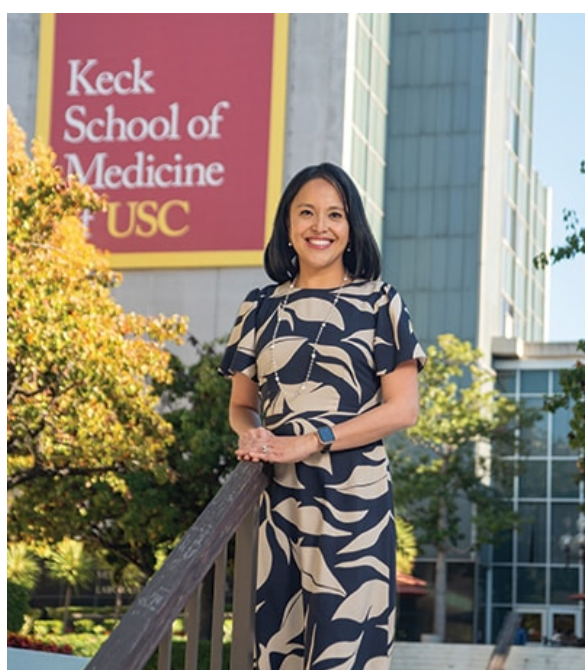
For her part, Dr. Bhargava, professor of pathology and laboratory medicine and senior laboratory director, University of California San Francisco, says that despite numerous publications that have shown band counting is unreliable and of questionable utility, “I was explaining, over and over again in CAP TODAY, the same thing.” Others on the committee—with its high representation of academic medical centers—had already stopped

reporting bands. “So the perception in the room was that the questions we were getting might represent a minority of folks. But we didn’t have formal data to back that up.”

Alas, they still don’t. Band counting was not, as it turns out, a rare artifact from a past era. “That was the biggest surprise—just the sheer number of people who are still reporting bands,” Dr. Bhargava says.

“Here we are,” agrees Dr. Vergara-Lluri, surveying the results and noting that a key article on the utility (or rather, lack of it) of band counting published three decades ago (Cornbleet PJ, et al. CAP TODAY. 1994;8[5]:37–41) should have solved the problem far sooner. Channeling her inner Stephen Sondheim, she adds, “We’re still here.”

This is not a glowing tribute to longevity. In the laboratory, “We all know this is not great,” says Dr. Vergara-Lluri, who is also medical section director of hematology and flow cytometry sections for the core laboratory of Los Angeles General Medical Center.



Band neutrophils were found to be commonly reported by a majority of surveyed laboratories. “It’s labor-intensive, it’s manual, it will delay turnaround time, and it siphons medical scientists’ resources” away from tests with greater clinical value and higher reproducibility, says Dr. Maria (Ria) Vergara-Lluri, at right. [Photo by Roland DeCrescent]

Multiple articles in the literature confirm that band counting is not reproducible. Moreover, she says, “It’s labor-intensive, it’s manual, it will delay turnaround time, and it siphons medical scientists’ resources” away from tests with greater clinical value and higher reproducibility.

That knowledge may be well known in the echo chamber of the laboratory community, Dr. Vergara-Lluri says, but it’s not widespread among clinicians, whose literature sometimes proposes a different story: that band counts are useful for sepsis evaluation. “Some pediatricians and hematology oncologists are still hanging on to the idea that this is essential.”

Dr. Vergara-Lluri recalls one study that suggested that a small apparent change in a patient's band neutrophils—say from three percent one day to seven percent the next—was clinically significant. But such studies can overlook the impact of Rumke intervals, the *Archives* study notes. As Dr. Vergara-Lluri explains, if a technologist counts seven percent bands in a 100-cell manual differential, the 95 percent confidence interval is two to 12 percent. "It's wide. We laboratorians understand that." But that nuance may be lost on clinicians, who may confer an unwarranted perfection on a laboratory result in the electronic medical record. "They think that seven percent is always going to be seven percent—today, tomorrow—and it means something when it jumps to 10 or even 12 percent," rather than falling within statistical probabilities.

At the same time, the authors knew they had to dig deeper into the practices of their laboratory colleagues, says Dr. Bhargava. Are they reporting percents? Absolute counts? Reference ranges? Are they reporting it in specific scenarios? At the behest of certain divisions like the NICU or hematology-oncology?

Secondly, Dr. Bhargava continues, while they knew some laboratories were still reporting band counts, "We also wanted to further drive home the point about its inaccuracy."

To do so they selected a peripheral blood smear from a neonate admitted shortly after birth for decreased movement and depressed respiratory rate with Apgar scores of three, seven, and eight at one, five, and 10 minutes; the most common clinical scenario for band count requests is neonatal sepsis, the authors note. The morphologic challenge included a dozen preselected cell identifications of segmented neutrophils, bands, and metamyelocytes, representing easy, moderately challenging, and difficult-to-classify bands.

In addition, they asked whether those performing band counts in the laboratory were specialists or generalists, and whether they performed counts manually or used an image analysis platform.

That led to another surprise. "It didn't matter," Dr. Bhargava says. "It was equally bad for everyone."

Given that interobserver variability in classifying a given cell is impacted by the way the nucleus may "kink" or "fold," it's "poor no matter what you do to try to improve it," Dr. Bhargava says.

No amount of training is likely to solve the problem. To those outside the laboratory, Dr. Vergara-Lluri says, the issues with identifying band neutrophils might seem to be related to technologists' skills. She's quick to disabuse folks of that notion. "Someone might say, 'You just don't train your techs well enough.' But they're very good at their jobs."

The authors did explore the hypothesis that laboratory training would make a difference. Would results be better in laboratories staffed by those trained only in hematology, for example, or those who performed higher volumes? "We asked all those questions," says Dr. Vergara-Lluri. There was no reproducible, consistent linkage between such factors and performance. "Which just means that it's hard to count bands."

Band neutrophils are by their nature difficult to identify. In moderate and difficult cell identifications, it's difficult to identify the filament that distinguishes between a segmented neutrophil and a band neutrophil. "You're not quite sure—I'm not seeing it but *maybe* it's there?" Dr. Vergara-Lluri says. "It's easy when it's easy, but when it's moderate and difficult, that's where the ambiguity rests."

If the nucleus has an identifiable thin filament connecting two or more lobes, Dr. Bhargava explains, then it's considered a segmented neutrophil. Anything short of that, she says, would be a band. But constrictions can pose a problem. "It lies in the eyes of the beholder whether the constriction is thin enough to be a filament, or is it really just a constriction."

Another problem lies with the manual nature of band counting. Most labs will perform a 100- or 200-cell differential, which leads to the wide statistical confidence interval of the count, says Dr. Bhargava; by comparison, an automated differential counts around 30,000 cells.

The survey also asked participants to perform a manual 100-cell differential on a single digital blood smear. “Even then the CV of the labs was really high—55.8 percent,” Dr. Vergara-Lluri says. One laboratory called a minimum of zero percent bands; another a maximum of 50 percent bands, on the exact same digitized smear. “That’s not very reproducible.”

What should laboratories do with this dispiriting data?

Dr. Vergara-Lluri sighs, then begins listing some options.

One tack would be to make it part of a laboratory stewardship initiative, she says, approaching the C-suite with a proposal to use the laboratory more wisely. With a shrinking workforce, and plenty of other manual demands in the hematology laboratory, “I don’t want our technologists spinning their wheels. Removing band counting would relieve the manual work that’s really not valuable,” Dr. Vergara-Lluri says.

“But I think we also have to have these conversations with our clinical colleagues,” she adds. “One way is to say, ‘I will provide you with quality care in tests that actually have good reproducibility, and we’ll improve your care. That’s not the case with band counts. It’s archaic, and we need to do something better and not tie up technologist time.’”

In her experience, hematology-oncology and pediatrician colleagues tend to be more conversant in the nuances of band counting. “They don’t use it,” Dr. Vergara-Lluri says, though she adds that the survey data indicated those two practices are a bit more likely to order it.

She’s encountered more pushback from generalists. She recently tried to persuade one clinical colleague to stop ordering CBCs with manual differentials to obtain a band count. “We know that having the manual differential tacked onto the CBC will increase turnaround times. Unfortunately, I hit a brick wall with this particular physician,” who insisted band counts were essential to identifying patients with sepsis. “No amount of data I could present would change their mind,” she says.

And that was just one physician. “I spend a lot of time thinking about this—how do we have this conversation on a large scale?” Dr. Vergara-Lluri says. As her interactions with that one colleague showed her, in some cases it’s a matter of changing hearts as well as minds. “There’s got to be a way to appeal to the emotional side or the logical side of a clinician’s brain to say, *We should not be doing this*. It’s a little shocking, to be honest. How are we still talking about this 30 years later?”

Tests, like facts, can be stubborn things. Dr. Vergara-Lluri compares band neutrophil counting to ESR—another test laboratories would like to drop. “But it lives on,” she says, as a favorite among rheumatologists. “I’ve personally given up on that one.”

Dr. Bhargava sees similarities to bleeding time. Though it was long known to lack sensitivity and specificity, “It took decades for people to finally stop doing it.”

It’s also possible that larger forces are keeping band counts in orbit.

The study noted that the majority of laboratories reported doing band counts with *all* their manual differentials. “So it wasn’t a clinical department holding out and saying, ‘I want that band count,’” Dr. Bhargava says.

A number of sepsis clinical assessment algorithms, such as the St. John sepsis protocol, incorporate band counting. It’s likely, says Dr. Bhargava, that clinical guidelines—“presumably formulated without the input of laboratorians, or made with old data”—have not been updated.

Clinicians may be unaware of the impact of inter- and intraobserver variability on these scores, Dr. Bhargava suggests. “You input a number, but it may not be indicating what you think it’s indicating. So it’s not simply that it’s labor-intensive and time-consuming, which it is, but it’s also not doing any good clinically.”

It's even possible, she adds, that clinicians would be equally surprised by the survey results. She offers a charitable take on the matter. "Maybe the labs that continue to report bands just haven't thought about it. It could be that they're just historically doing this, and their own clinicians may not even be using it anymore."



Dr.
Pozdnyakova

Tradition can indeed be a culprit, says coauthor Olga Pozdnyakova, MD, PhD, associate pathologist, Brigham and Women's Hospital; medical director, Mass General Brigham flow cytometry integrated laboratory service; medical director, Brigham and Women's hematology laboratory and Harbor Medical Physician Diagnostics Laboratory; and associate professor, Harvard Medical School.

"For pathologists, it's sometimes hard to influence the clinical community," Dr. Pozdnyakova says. If not quite a battle of the bands, making a change does mean pathologists will have to confront a persistent chorus: *This is how it has been done traditionally*. In her own experience, she says, some clinical specialists "do not believe me. Because it's always been done like that."

Nevertheless, the authors express hope that the *Archives* article will be the jolt they need to reach out to clinical teams. Dr. Pozdnyakova says, "Now we actually have data from the study that show counting of bands is not appropriate, and it is time for a change."

About a half dozen years or so ago Brigham and Women's stopped reporting bands separately; now, they're counted together with segmented neutrophils. Before the change, the laboratory did not include bands in the absolute neutrophil count. Explains Dr. Pozdnyakova: "Bands were not considered to be mature neutrophils, which is incorrect. So in several instances we would report a falsely low neutrophil count."

"It does have significant implications if it's not assessed properly," she continues. Physicians don't necessarily know the intricacies of what's considered a mature versus a nonmature neutrophil. "All they see is a normal white blood cell count and a low absolute neutrophil count," which can send them chasing after more testing.

Dr. Pozdnyakova instituted the change to combine bands and neutrophils at Brigham and Women's, using an approach that set fire to the usual, play-nicely playbook.

Asked how she achieved what was essentially a bloodless coup, she laughs, then says, "Well, to tell you the truth, we just sent a broadcast saying that because bands are considered to be mature neutrophils, the absolute neutrophil count will now be reported together with segmented and band neutrophils."

And the response? "No one has noticed," she says. You can almost hear the smile in her voice. "That was the best way. No one had questions."

This unilateral move was unusual, she concedes. The size of her institution may have helped. "We always complain that sometimes the bigger the institution is, the more difficult it is to make a change. But I think when you are 100 percent confident your decision is correct, it's almost easier to make that change because you don't have to go to every single person—because there are tens of thousands of us." A smaller institution might require more personal outreach, she says.

Occasionally, Dr. Pozdnyakova says, she received an email asking about the reasons for the change. "And then you'll explain it to them, and they'll be perfectly fine with it."

Neonatology is, perhaps unsurprisingly, the exception. “Unfortunately,” she says. “They’re still asking us to perform band counts. Because neonatologists still swear by bands. When it concerns little babies, it’s really difficult to make changes. But we can start with grownups.”

How hard will it be to turn that particular tide? “My husband is a neonatologist at [Massachusetts General Hospital], and I cannot convince him,” Dr. Pozdnyakova says with a laugh. “If I cannot convince him, then I will have trouble convincing other people.”

Band counting isn’t performed at UCSF. The practice was discontinued there before Dr. Bhargava arrived six years ago.

But in her previous position in Boston, she was part of an effort to make the change. Much of it was driven by presenting data to clinical groups, she says.

Neonatal ICU physicians were the most resistant, she recalls, in part because there are few other parameters they can use for this patient population. “For a while we allowed them as an exception, and they could specifically ask for a band count. And then we slowly helped them transition.”

Many NICU physicians were accustomed to using the I:T ratio, or immature to total neutrophils, which requires band enumeration. Those who learned this as part of their training, Dr. Bhargava says, also tended to be more resistant to change.

But persistence paid off. Likewise, for laboratories ready to make a change, Dr. Bhargava urges pathologists to reach out to key groups. “Tell them there has been decades of data and papers that tell us how bad the variability is in band counting.”

Does an alternative test exist? Ideally, it would perform well, have a fairly tight confidence interval, and isn’t costly. (“Band counting is none of those things,” Dr. Vergara-Lluri says with a laugh.) The *Archives* article alludes to several promising possibilities, including immature granulocytes, C-reactive protein, and procalcitonin, though the literature in this field needs to grow.

“None are perfect,” Dr. Bhargava says. “No one CBC parameter is great at predicting sepsis. The bottom line is, if bands are of low added value and so are IGs, pick the one that is more automated and less time-consuming.”

And while Dr. Pozdnyakova’s maverick approach may not work everywhere—not everyone feels comfortable trading a three-point turn for a U-turn into oncoming traffic and then hitting the gas—laboratories do need to tackle the problem. “If every one of us starts in their own institution, I think we’ll achieve great results,” Dr. Pozdnyakova says.

“It sounds like a long road ahead,” agrees Dr. Vergara-Lluri, “but I’m hopeful.”

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