Q&A column, 3/17

Editor: Frederick L. Kiechle, MD, PhD

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Submit a Question

Q. Our hospital system is implementing Sysmex instruments with a focus on the accuracy of the absolute white blood cell values—use of the absolute neutrophil count and immature granulocytes with the WBC as markers for septicemia. I then became aware that the hospital purchased the St. John Sepsis v14 protocol, which lists 10 percent bands as one of the markers for septicemia. The Rumke for 10 percent is 4–16. Using bands is not consistent with reducing manual differentials and is not an accurate parameter to use. Are there other protocols using WBC/ANC?

A. Sepsis is a syndrome of organ dysfunction related to underlying infection. There are several clinical assessment tools for sepsis evaluation. In most instances, CBC assessment of leukopenia (12 k/µL) is among the signs used in the screening of sepsis (www.bit.ly/SepsisScreen). This is based on the 1991 Systemic Inflammatory Response Syndrome (SIRS) criteria, which also includes >10 percent bands as a criterion. There are conflicting data on the utility of band count in evaluating sepsis. While some studies have shown that a subset of patients with culture-proven sepsis may have a normal WBC count but elevated band count (Seigel TA, et al. *J Emerg Med.* 2012;42[3]:254–259), others have shown that band counts are unhelpful in evaluating sepsis in patients with normal WBC counts (Mare TA, et al. *Crit Care.* 2015;19:57) or have little added benefit when ANC and presence of cells more immature than bands are taken into consideration (Ardron MJ, et al. *Am J Clin Pathol.* 1994;102[5]:646–649).

Reporting band counts necessitates a manual differential, which increases costs and turnaround time. Additionally, there is poor interobserver agreement for identification of bands (van der Meer W, et al. *Eur J Haematol.* 2006;76[3]:251–254). Add to this the lack of accuracy in a 100-cell differential, as referenced by the reader, and this result is inherently unreliable. A 1992 CAP TODAY Q&A (6[4]:65–66) by Thomas F. Dutcher, MD, on the significance of bands argued that bands should not be reported separately. Joan Etzell, MD, also discussed this in the November 2010 issue of CAP TODAY (24[11]:54–60).

Subsequently, many laboratories have altogether stopped the practice of reporting bands. Given that bandemia continues to appear in some adult and neonatal sepsis evaluation clinical protocols, stopping the reporting of bands would require a dialogue with clinical colleagues. Several of the sepsis screening tools available on the Surviving Sepsis Campaign website (www.bit.ly/SepsisResources) do not list band percentage as a criterion.

In the laboratory of the Beth Israel Deaconess Medical Center, the default is to run automated differential counts and, in the absence of other flags, reflex to manual differential only when immature granulocyte counts are greater than two percent (we are evaluating a higher threshold of five percent) on our Sysmex XN platforms. In reviewing our validation studies, automated immature granulocyte counts have a positive bias in detecting non-band immature granulocytes compared with manual counts. Therefore, we are unlikely to miss a true left-shift by defaulting to an automated differential count. Automated immature granulocyte (IG%) counts have been assessed in some adult and pediatric patients and were found to be of utility in sepsis assessment (Nierhaus A, et al. *BMC Immunol.* 2013;14:8; van der Geest PJ, et al. *J Crit Care.* 2014;29[4]:523–527).

In the "Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)" published in February 2016 (Singer M, et al. *JAMA.* 2016;315[8]:801–810), sepsis is defined as "life-threatening organ dysfunction caused by a dysregulated host response." This definition emphasizes the task force's opinion that the "nonhomeostatic

host response" to infection is the aspect of sepsis that makes it more dangerous than simple infection alone. The guidelines indicate that SIRS criteria such as neutrophilia are indicators of infection, but that they may be physiologically appropriate and are generally nonspecific to sepsis. Instead they recommend using a ≥ 2 points change from baseline SOFA score (Sequential Organ Failure Assessment; based on respiratory rate, platelet count, bilirubin, MAP/pressor dosage, Glasgow Coma Scale score, creatinine/urine output), which was highly predictive for sepsis in ICU patients. For patients not in the ICU, any two quickSOFA (qSOFA) criteria were predictive (respiratory rate ≥ 22/min, altered mentation, systolic blood pressure ≤ 100 mm Hg). Although these criteria do not include WBC, neutrophil, or band count, it should be noted that the guidelines were based on a retrospective cohort study of patients from 12 community and academic hospitals who had "suspected infection" defined as a combination of antibiotic administration and body fluid culture (Seymour CW, et al. *JAMA.* 2016;315[8]:762–774). WBC, neutrophil, or band count may have influenced the initial suspicion for infection in these cases, but this effect cannot be measured given this study design.

Hospitals and health care systems are increasingly leveraging the power of their electronic health record systems to provide clinical decision support (CDS) tools to avoid missing diagnoses or actionable items. For example, the St. John Sepsis v14 protocol is a commercially available algorithm offered by Cerner as an adjunctive software component to Cerner Millennium. Compared with traditional ward-specific, paper-based screening tools, these clinical decision supports provide more standardization, a capacity for constant upgrading and revision, and a level of "digital surveillance" over the patient's aggregated clinical, radiologic, and laboratory results. Ultimately, the decision of whether to use a CDS, and which one to use, will likely be related to the institution's EHR. Ideally, software vendors will update these algorithms as new guidelines, such as Sepsis-3, emerge. In the meantime, Cerner's website indicates that threshold values on the algorithm can be changed; other clinical decision support may be similarly customizable. Given the concerns with the methodological veracity and clinical significance of band count, laboratorians can reasonably advocate for removal of this criterion from clinical decision support tools.

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Q. We are seeing more bilateral salpingectomies as sterilization procedures and need guidance on sampling and diagnostic lexicon. The poster child is a 50-year-old woman who had a total abdominal hysterectomy with bilateral salpingectomy for myomata where a modest 0.5 mm atypia in a fimbria of fallopian tube (a chance encounter) was a minor cytologic change and was promoted to serous tubal intraepithelial lesion. That led to subsequent bilateral oophorectomy (all tubal tissue was put through, was p53 positive, and so on). I believe* BRCA *risk-reducing bilateral salpingo-oophorectomies are supposed to have a specific consent about the experimental nature of doing pathology that includes complete sectioning. What are appropriate sampling and diagnoses for bilateral salpingectomies performed for sterilization?

A. Recent research studies suggest that the fallopian tube may be the site of origin of some pelvic high-grade serous carcinomas. In patients with *BRCA1* and *BRCA2* germline mutations, who are at increased risk of developing ovarian carcinoma, studies have shown that prophylactic salpingo-oophorectomy has reduced the risk of developing ovarian carcinoma. Given the data suggesting the fallopian tube as the site of origin of many pelvic serous carcinomas, it has been proposed that prophylactic salpingectomy, even in non-high-risk (average risk) patients, may serve to reduce the incidence of ovarian carcinoma. Thus in 2013, the Society of Gynecologic Oncology presented a clinical practice statement stating that if women at average risk of developing ovarian cancer undergo a hysterectomy, sterilization procedure (tubal ligation), or other type of abdominal or pelvic surgery, and have completed childbearing, bilateral salpingectomy should be considered as a risk-reducing

procedure.

For example, in an average-risk patient who wants to undergo a sterilization procedure, instead of doing a standard tubal ligation, the current SGO recommendation is for there to be discussion about and consideration of risk-reducing bilateral salpingectomy. The rationale is to reduce the risk of developing pelvic high-grade serous carcinoma, given the theory that a number of such tumors originate from the fallopian tube. For this reason, many gynecologists are beginning to increasingly perform complete salpingectomies in lieu of tubal ligations in patients undergoing sterilization, after careful discussion with their patients regarding the potential benefits of removing the fallopian tube completely.

With regard to sampling complete salpingectomy specimens that are received for sterilization, the SGO practice guideline states that specimen sampling should include entire sectioning and microscopic examination of the fimbriae and representative cross-sections of the remainder of the fallopian tube, as well as any other suspicious lesions if present. Thus, unlike in high-risk patients (*BRCA1* and *BRCA2* carriers) where the entire fallopian tube is examined, typically per the SEE-FIM protocol (Sectioning and Extensively Examining the FIMbria), in average risk patients only the fimbriated end needs to be submitted entirely. Representative cross-sections of the tube should also be submitted. It is recommended that the fimbriae be sectioned longitudinally, per the SEE-FIM protocol. Longitudinal (lengthwise) sectioning provides maximum exposure of the tubal plicae. It is important to examine the fimbriae in their entirety, as they are the most likely site of occult carcinoma in the fallopian tube, most often being serous tubal intraepithelial carcinoma (STIC).

The additional sampling of the fallopian tube in salpingectomy specimens for sterilization does not change the standard way reporting such specimens is performed. If there are any pertinent pathologic findings, those still need to be reported, as before. Stating pertinent negative findings in a microscopic description, such as “no malignancy identified,” is also useful. In most cases, the fallopian tube diagnosis will entail stating that no diagnostic pathologic change was seen.

Doing additional immunohistochemical studies, such as p53 and MIB-1, routinely on every case is not recommended. Performing such immunohistochemical studies should be limited to cases in which there are morphologic changes on hematoxylin and eosin (H&E) stain that warrant further investigation, such as significant cytologic atypia, loss of polarity, and associated mitotic activity. An important factor to keep in mind is that normal fallopian tube epithelial cells at baseline can have a certain degree of variation in nuclear morphology.

In cases where there are cytologic abnormalities that are concerning on H&E stain, immunohistochemical stains such as p53 and MIB-1 may be performed in order to evaluate the possibility of a STIC lesion. STIC lesions are characterized by the following features: 1) increased nuclear to cytoplasmic ratio, with more rounded nuclei; 2) loss of cell polarity; 3) prominent nucleoli; and 4) absence of ciliated cells. Additional findings include epithelial stratification, small fracture lines in the epithelium, and exfoliation from the tubal surface of small epithelial cell clusters, with or without degenerative changes. The cells have strong and diffuse p53 nuclear staining in greater than 75 percent of cells, or have completely negative staining (null-phenotype). The MIB-1 index is greater than 15 percent and can exceed 50 percent.

With increased examination of the fallopian tube, as well as increased use of immunohistochemistry, a variety of additional lesions have been described that range from histologically normal-appearing tubal epithelium that overexpresses p53 (p53 signature) to lesions with cytological atypia that do not meet the diagnostic threshold of STIC. P53 signature is characterized by foci of at least 12 consecutive morphologically benign, p53-positive secretory cells with a low MIB-1 proliferation index. Additionally, “serous tubal intraepithelial lesion,” “tubal intraepithelial lesion in transition,” and “serous tubal intraepithelial neoplasia,” among others, are terms that have been variably used to describe lesions with p53-positive foci with cytologic features intermediate between p53 signatures and STIC. Additional study is needed to fully refine diagnostic criteria for these atypical intermediate lesions, as their reproducibility and clinical significance are still uncertain. In clinical practice, such atypical lesions can be given a descriptive diagnosis, and a comment can be made that the lesion is insufficient for a diagnosis of STIC. “P53 signature” is not recommended as a diagnostic term in a pathology report, as the clinical significance is

also still uncertain.

The Society of Gynecologic Oncology clinical practice statement regarding salpingectomy for ovarian cancer prevention is available at www.bit.ly/SGO-ovcancer.

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