Effects of Yeast on Automated Cell Counting

John A. Branda, MD, and Alexander Kratz, MD, PhD, MPH

Key Words: Cell counters; Fungemia; Candida; Yeast; Hematology analyzers; ADVIA 120/2120; Automated cell analysis; Flow cytometry

DOI: 10.1309/1G5HB7PAK8V0KAR7

Abstract

We studied the effects of yeast in peripheral blood samples on results reported by an ADVIA 120/2120 Hematology System (Bayer HealthCare, Diagnostics Division, Tarrytown, NY). In a simulated candidemia model, very high concentrations (1-5 × 10⁸ colony-forming units [CFU]/mL) of Candida glabrata and Candida parapsilosis caused a spuriously elevated platelet count. No such effect was observed with Candida albicans. All 3 yeast species, when present at a concentration of 1-5 × 10⁶ CFU/mL or greater, increased the automated WBC counts significantly and in a dose-dependent manner. The yeast cells were mainly misidentified as lymphocytes. All spurious results were flagged by the cell counter for microscopic review. We conclude that although the presence of yeast in a blood sample can interfere with the ADVIA 120/2120 Hematology System, compromised results are appropriately flagged by the instrument and are seen only when the yeast concentration is very high.

Automated cell counters have a central role in the hematology section of modern clinical laboratories. State-of-the-art instruments can analyze large numbers of cells in a short time (often measured in seconds) with higher accuracy and reproducibility than microscopy and at a fraction of the cost of manual methods. Therefore, the vast majority of results reported from most modern hematology laboratories are released directly from automated instruments, without microscopic review of a blood smear. Most laboratories perform a microscopic examination only on samples flagged by the automated cell counter as requiring a manual review.

Although it is generally agreed that automated cell counters have improved the quality of routine blood cell analysis, a number of potential interfering factors have been identified for these instruments, including spuriously elevated platelet counts caused by microspherocytosis or by bacteremia.1-3 Arnold and coworkers4 and Latif and colleagues5 reported that Candida glabrata fungemia can lead to a falsely elevated platelet count on the H*2 (Bayer HealthCare, Diagnostics Division, Tarrytown, NY) and on the Cell-DYN 4000 (Abbott Diagnostics, Santa Clara, CA) hematology analyzers in thrombocytopenic patients.

In the case reported by Latif et al,5 the Cell-DYN 4000 analyzer had not generated any platelet flags despite the spurious nature of the platelet count. These findings are of concern because many patients are at dual risk for thrombocytopenia and fungemia, and the reporting of a falsely high platelet count in a thrombocytopenic patient can have potentially serious clinical consequences. The findings described by Arnold and colleagues4 and Latif et al5 were based on individual cases, and the authors did not report the quantity of yeast necessary to influence results derived from an automated cell counter.
Consequently, it is uncertain whether one should expect yeast interference to be a common or rare event. The yeast interference described by Arnold et al also involved a cell counter that has since been replaced by newer generation models.

We systematically studied the effect of yeast in blood on cell counting using a state-of-the-art automated hematology analyzer, the ADVIA 120/2120 system (Bayer HealthCare). We simulated yeast fungemia by adding known concentrations of yeast to blood from cytopenic patients and analyzed the specimens with the cell counter. The yeasts chosen were Candida species because as a group, they represent the most common fungal cause of nosocomial bloodstream infection. We chose Candida albicans, C. glabrata, and Candida parapsilosis in particular because these are the most common bloodstream isolates among the Candida species according to recent surveillance data. C. glabrata also was the cause of the platelet-counting interference reported by Arnold et al and Latif et al.

Materials and Methods

Study Design

To determine the effect of yeast fungemia on automated cell counting, we added increasing amounts of yeast (C. albicans, C. glabrata, or C. parapsilosis) to aliquots of blood. The aliquots were analyzed within an hour by a laboratory technologist using an ADVIA 120 Hematology System.

Blood Samples

The blood samples used for this study were discarded remnants of samples obtained for clinical purposes for CBC and/or WBC differential counts that had been collected into BD Vacutainer K$_2$EDTA Plus plastic tubes (Becton Dickinson, Franklin Lakes, NJ). We used 3 neutropenic (WBC count, <500/µL [<0.5 × 10$^9$/L]) and thrombocytopenic (platelet count, <30 × 10$^3$/µL [<30 × 10$^9$/L]) samples and 1 sample with normal counts for each of the 3 species of yeast in spiking experiments, for a total of 4 blood samples per species. The use of samples with low WBC and platelet counts was meant to simulate a frequent clinical situation in fungemic patients, many of whom are cytopenic from chemotherapy.

Yeast

Isolates of C. albicans, C. parapsilosis, and C. glabrata were acquired from the American Type Culture Collection (ATCC Nos. 14053, 22019, and 15126; Manassas, VA). Highly turbid saline suspensions were prepared from overnight cultures by picking numerous colonies and suspending them in sterile 0.85% sodium chloride by use of an electric vortex. Serial 10-fold dilutions were prepared from the undiluted stock suspensions, with three 10-fold dilutions in addition to the stock suspension per yeast strain. The cell density of the stock suspensions was adjusted so that the third and final 10-fold dilution matched a 0.5 McFarland turbidity standard (Remel, Lenexa, KS), using a turbidity meter (A-Just, Abbott Laboratories, North Chicago, IL). Given that yeast suspensions adjusted to a 0.5 McFarland standard contain approximately 1-5 × 10$^8$ yeast cells/mL, the undiluted stock suspensions therefore contained approximately 1-5 × 10$^9$ cells/mL in the first tube, approximately 1-5 × 10$^7$ cells/mL in the second tube, and approximately 1-5 × 10$^6$ cells/mL in the third tube. The yeast concentration of each undiluted stock suspension and of each 10-fold dilution was verified by colony counts on Sabouraud dextrose agar to determine the colony-forming units (CFU) per milliliter (data not shown).

Simulated Fungemia

For each of the 3 Candida species, 4 test mixtures plus a negative control sample were prepared from each blood specimen used. In each case, 1 aliquot represented the negative control sample, containing no yeast. The other 4 aliquots contained approximately 1-5 × 10$^5$, 1-5 × 10$^6$, 1-5 × 10$^7$, and 1-5 × 10$^8$ CFU/mL, respectively, and were prepared using the yeast suspensions described in the preceding section.

Cell Counter

Samples were analyzed on an ADVIA 120 Hematology System in the CBC/DIFF mode. The underlying technology of this instrument, a part of the ADVIA 120/2120 series, was reviewed recently. Briefly, RBCs and platelets are analyzed using isovolumetric spherical and light scatter. Low-angle (2°-3°) and high-angle (5°-15°) light scatter signals are converted into volume and refractive index values using the Mie theory of light scatter for homogeneous spheres. For RBCs, the light scatter signals correspond to cell volume and hemoglobin concentration. For platelets, they provide platelet volume and platelet component concentration.

Two channels are used to analyze WBCs: a peroxidase channel, in which a peroxidase reagent and light scatter are used to differentiate WBCs by myeloperoxidase content and size, and a lobularity/nuclear density channel (also known as the basophil channel), in which differential WBC lysis is combined with light scatter analysis to determine WBC counts, differential data, and information about nuclear maturity of WBCs. The WBC count for each sample is determined independently in the lobularity/nuclear density channel and the peroxidase channel. The 2 results are compared automatically by the instrument, and if they differ by more than a preset limit, a “comparison error” flag is triggered, and the WBC count is flagged, necessitating preparation of a smear for microscopic review. If the 2 results are within the preset limit, the WBC count from the lobularity/nuclear density channel is released by the instrument.
Analyzers were operated by hospital technologists according to standard laboratory procedures. Samples were coded, and technologists were blinded as to the meaning of the codes. Calibration of the instruments is performed twice per year using the ADVIA SETpoint calibrator, according to the manufacturer’s instructions. Manufacturer-supplied controls are run on every shift at 3 levels (high, normal, and low) for CBC and differential parameters. Flagging criteria (ie, the conditions that will cause a sample to be flagged by the analyzer and a smear to be prepared for microscopic review) had been set to conform with the manufacturer’s recommendations and the clinical needs of the patient population at our institution.

**Results**

The presence of yeast in blood samples did not affect RBC counts on the ADVIA 120, regardless of yeast concentration or species (data not shown). The effect of yeast on platelet and WBC counts are shown in **Figure 1**. *C. albicans*
had no effect on the platelet count, even at the highest concentration assayed (~1-5 × 10⁸ CFU/mL). *C glabrata* and *C parapsilosis* did not influence the platelet count at concentrations up to and including 1-5 × 10⁷ CFU/mL; at 1-5 × 10⁸ CFU/mL, however, these 2 species caused a significant increase in the platelet count. All samples showing a falsely elevated platelet count owing to the presence of yeast were flagged by the cell counter for the presence of platelet clumps.

Each of the *Candida* species, when present at concentrations of 1-5 × 10⁶ CFU/mL or greater, caused a significant increase in the WBC count obtained in the lobularity/nuclear density channel for all samples tested. An increase in the WBC count also was observed in the peroxidase channel. The effect of candidemia on the WBC count in the peroxidase channel was present mainly at concentrations of 1-5 × 10⁷ CFU/mL and greater, however, and at a given yeast concentration, it was only a fraction of the effect seen in the lobularity/nuclear density channel. Because of the discrepant effect on the 2 WBC channels, the instrument flagged a comparison error (WBC-CE) for the WBC parameter for all samples with falsely elevated WBC counts. Figure 2 and Figure 3 show examples of the cytograms generated by the lobularity/nuclear density and the peroxidase channels of the cell counter. As the amount of yeast present in the samples increased, the number of signals misinterpreted as WBCs increased commensurately. The majority of these spurious signals were misidentified by the instrument as lymphocytes because they were small and peroxidase-negative. Figure 4.

**Discussion**

By using an in vitro candidemia model, we systematically studied the effect of *C albicans*, *C glabrata*, and *C parapsilosis* on CBC and differential results obtained by the ADVIA 120/2120 Hematology System. We found that yeast affected platelet counts only at the highest concentrations tested (1-5 × 10⁸ CFU/mL), and the interference was limited to *C glabrata* and *C parapsilosis*. *C albicans*, the yeast species most frequently isolated from blood, had no effect on platelet counts, even at a concentration of 1-5 × 10⁸ CFU/mL. The ADVIA 120/2120 Hematology System uses low- and high-angle light scatter to identify platelets by volume and refractive index. Therefore, it can be hypothesized that the differences produced by the yeast species observed in our study are due to disparities in their light-scattering properties.

Our findings match those of Arnold and colleagues and Latif et al, who described in case reports platelet-counting interference by *C glabrata*. Latif and colleagues also reported that suspensions of *C glabrata* showed optical scatter patterns similar to those of platelets, whereas suspensions of *C albicans* and *C parapsilosis* yielded optical scatter plots that did not resemble those of platelets.

Automated platelet counts can be obtained by optical methods (based on light scatter or fluorescence, eg, the ADVIA 120/2120 Hematology System and the H*2); by electrical impedance (the “Coulter Principle,” eg, the LH-750,
Beckman Coulter, Fullerton, CA; and the Pentra 120, Horiba ABX, Montpellier, France); or by a combination of optical and impedance measurements (eg, the Cell-DYN 4000 and the XE-2100, Sysmex, Long Grove, IL). In addition, the Cell-DYN 4000 has the ability to provide an immunoplatelet count with anti-CD61 antibodies. Our study and the report by Arnold and colleagues address the effect of yeast on instruments based on light scatter. The instrument used by Latif and colleagues uses optical and impedance methods to identify platelets, and both methods incorrectly identified the yeast forms as platelets. Latif and coworkers also indicated that analysis of saline suspensions of C glabrata (but not of C albicans or C parapsilosis) demonstrated patterns of electrical impedance (and light scatter) similar to those of platelets. To our knowledge, no studies have specifically addressed the possibility of yeast interference with instruments using only the impedance method. Based on the observations of Latif and coworkers, it is possible to speculate that platelet counts based solely on impedance also are subject to spurious results caused by yeast. Further studies of this question are clearly needed.

It is important to note that a platelet flag was generated by the ADVIA 120/2120 system whenever the platelet count was elevated spuriously by the presence of yeast. This is in contrast with the case reported by Latif and colleagues, who saw no flagging of their patient’s spuriously elevated platelet count. (Arnold and coworkers did not comment on whether their cell counter flagged the platelet count of their patient.) Differences in the underlying technology (such as the light angles analyzed and the algorithms used by the instruments) are the most likely explanation for the ability of the ADVIA 120/2120 system to flag spuriously elevated platelet counts, in contrast with the platform used by Latif and coworkers. The generation of a platelet flag should prevent medical error in cases in which high-grade candidemia leads to platelet interference on the ADVIA 120/2120.

All 3 Candida species had a significant effect on the WBC count in our simulated candidemia model. Yeast forms were misclassified mainly as lymphocytes by the cell counter, and the interference was seen in cytopenic blood samples and blood samples with normal cellular parameters. The effect was most pronounced in the lobularity/nuclear density channel of the instrument, but also was present to a lesser degree in the peroxidase channel. The relationship between yeast concentration and WBC count was roughly linear, starting with a modest increase in baseline WBC count at a yeast concentration of 1-5 × 10^6 CFU/mL and increasing from there in a dose-dependent manner.

This spurious elevation of the automated WBC count by yeast interference causes concern because of its potential clinical consequences. Neutropenic patients are at increased risk of systemic fungal infections, and empiric antifungal therapy often is a consideration in febrile patients whose total WBC count is below a certain threshold. If a cytopenic patient with candidemia is erroneously assigned a total WBC count above the threshold, caregivers may withhold necessary and potentially lifesaving antifungal therapy. Fortunately, our study showed that all samples for which a spuriously elevated WBC count was generated were flagged by the instrument for a WBC-CE (ie, the WBC counts obtained in the lobularity/nuclear density channel and the peroxidase channel differed by more than the preset limit). When the WBC-CE flag is triggered, the WBC count and proportional and absolute differential results also are flagged automatically. Had these been clinical specimens, they would have been reviewed microscopically and the WBC count would have been corrected.

Unlike the ADVIA 120/2120, some automated cell counters use only 1 channel to count WBCs and, therefore,
cannot compare 2 independently obtained WBC counts. Further studies will be needed to determine whether the presence of yeast in peripheral blood samples can lead to a spurious elevation of WBC counts on other automated cell counters and whether those cell counters provide appropriate flagging of the WBC parameter.

Our findings demonstrate the possibility of spurious changes in CBC parameters caused by yeast concentrations of 1-5 × 10^6 CFU/mL and higher. In the cases reported by Arnold and colleagues and Latif and coworkers, yeast interference falsely increased platelet counts by 22 to 29 × 10^9/μL (22-29 × 10^9/L). Assuming that the automated cell counters misidentified and miscounted every yeast form as a platelet, this would translate to a fungal load (yeast concentration) of 2.2 to 2.9 × 10^7/mL, consistent with our findings.

A review of the literature, however, shows that such high yeast concentrations must be outliers. Studies using quantitative culture have documented a yeast concentration of 100 CFU/mL or less in the majority of fungemia cases. Fungal loads higher than 100 CFU/mL, however, usually are not quantified by this method because the colonies are too numerous to count (confluent growth). Such high levels are instead expressed semiquantitatively as 10 CFU/mL or more or 100 CFU/mL or more, and, therefore, it is not possible to know the exact concentration of yeast in samples classified as high-grade fungemia. By using a polymerase chain reaction–based assay, Maaroufi and colleagues found a wide range of C. albicans loads among 11 positive samples tested, ranging from 5 to 100,475 CFU/mL. The second highest fungal load in their study was 3,760 CFU/mL. Taken together, these studies suggest that fungal loads of 1-5 × 10^6 CFU/mL (the lowest concentration at which we saw an effect on CBC results) are unusual.

We demonstrated a cause-and-effect relationship between the presence of yeast and the induction of changes in automated blood cell count parameters. Although the presence of yeast can influence the platelet and the WBC counts generated by the ADVIA 120/2120 Hematology System, the spurious results will be flagged by the analyzer for manual review. Therefore, the CBC and WBC differential parameters directly released from an ADVIA 120/2120 Hematology System are unlikely to be influenced by the presence of yeast in a sample. Furthermore, yeast interference is seen only when the yeast concentration is unusually high. Technologists and pathologists reviewing flagged blood smears should be alert to the possibility that a flag can be triggered by candidemia and should have a low threshold for performing manual cell counts if they identify yeast on a blood smear with a flagged parameter.

References


From the Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston.


