Blood Developments

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DETECTION OF BACTERIAL CONTAMINATION IN PLATELET COMPONENTS

Extensive donor selection and testing strategies effectively reduce the transmission of viral agents by asymptomatic volunteer blood donors. Today, the most frequent transfusion-associated infectious risk in the United States is sepsis associated with bacterial contamination of platelet components.

Given the frequency, clinical significance, and potential for fatal complications that the presence of bacteria poses for certain patients, two accrediting agencies (the American Association of Blood Banks and the College of American Pathologists) have issued directives requiring the use of methods to detect and limit bacterial contamination of platelets.

CONTAMINATION IN PLATELET COMPONENTS

While bacterial contamination may affect any blood component, the ambient storage temperature conditions for platelets make them most likely to facilitate bacterial growth. Bacterial contamination of platelets can be found in about 1 in 1,000 to 1 in 3,000 units (depending on the source of the component and the methodology used to detect contamination). Bacteria, and/or the endotoxins they produce, are introduced into the circulation in amounts sufficient to cause sepsis or endotoxic shock. Estimates of the incidence of clinically significant complications related to this problem remain imprecise, due to the variability in case definition, protocols to evaluate transfusion reactions, and the methods used to detect bacteria in blood components.

In addition, many instances of clinically significant platelet bacterial contamination are neither recognized, nor reported. In a multi-center study coordinated by the US Centers for Disease Control and Prevention (CDC) designed to identify and confirm bacterial contamination in transfused blood components (the "BaCon Study"), the incidence of clinically significant platelet associated contamination was estimated at 1/100,000 and death at 1/500,000 platelet transfusions.[1] However, the report's authors acknowledged that the strict inclusion criteria for the study and the voluntary manner of reporting likely resulted in substantially underestimating the incidence of this problem. In addition, some contaminated platelet infusions go unrecognized because the patients are receiving antibiotic therapy for their underlying condition. Other investigators have estimated that 1 in 2,500 to 11,400 whole blood derived platelet pools and 1 in 15,400 apheresis platelet transfusions result in a clinically significant reaction.[2,3]

Although the concentration of bacteria required to cause clinically significant reactions varies depending on the microorganism, endotoxin production, clinical condition of the recipient, and other factors, it is known that as few as 10^2 to 10^3 CFU/mL have been associated with fever and positive blood cultures.[4]

SUMMARY

- Bacterial contamination of platelets represents the most frequent transfusion-associated infectious risk.
- Bacterial contamination levels of as few as 10^2 to 10^3 CFU/mL have been associated with fever and positive blood cultures.
- The incidence of clinically significant platelet-associated contamination is estimated at 1/15,000 and death due to sepsis or endotoxemia at 1/60,000 platelet transfusions.
- New measures to limit and detect bacterial contamination in platelet components to be implemented by March 2004 are expected to significantly decrease current risks.

SOURCES OF CONTAMINATION

Inoculation of bacteria in the blood collection set may result from donor bacteremia or the skin surface during phlebotomy procedure. The latter is believed to be the most common source, since the majority of the isolates are microorganisms found on the skin and in dermal appendages. The deeper epidermal layers, hair follicles, and sebaceous glands harbor bacteria that are difficult to remove, even after thorough mechanical and antiseptic arm preparation.

Although skin flora are the most common bacteria, blood collection from asymptomatic, transiently bacteremic donors is involved in a disproportionate share of cases of septic deaths associated with platelet transfusions. Lethal outcomes result from both bacterial growth and endotoxin production during storage. Most of the donor bacteremias involve Gram negative microorganisms.

PREVENTIVE STRATEGIES

Proper aseptic technique during phlebotomy is the first line of defense in preventing bacterial contamination of blood components. The use of skin disinfectant solutions that provide maximum bactericidal effect (e.g., iodophors or chlorhexidine) is recommended,[5] but recognized as only one measure in the prevention of bacterial entry into the blood unit. A promising intervention consists of the diversion of the initial aliquot of donor blood into an integrally connected pouch. This reduces the possibility that a skin plug or core cut with the needle during phlebotomy, harboring bacteria, will contaminate the collection bag. Blood in the diversion pouch can be used for immunohematologic and viral marker testing, without increasing blood loss associated with donation. Laboratory and actual experience in European centers indicate that this change in blood collection technique [diversion pouch] may reduce bacterial contamination rates in blood components overall by about 40%, with the highest reduction observed for common skin contaminants of the Staphilococcus family.[6]

BACTERIAL DETECTION

Detection of bacterial contamination prior to transfusion, even if costly, would be a significant step towards the elimination of transfusion-associated sepsis. The ideal characteristics of a bacterial detection method are that it be simple, practical, rapid, sensitive, specific, and inexpensive.

At this point, two culture-based methods for bacterial detection have been licensed by the Food and Drug Administration (FDA) to test platelet components "for quality control purposes." (FDA requires additional data to permit use of these devices for "donor screening," which implies an additional safety claim.) One of the quality control devices, intended for use with leukoreduced platelets collected by apheresis only, detects bacterial growth by sensing $CO2$ production. The second method relies on oxygen consumption for the detection of bacterial growth, and has been approved for both platelets from whole blood and apheresis. Oxygen consumption measurement limits detection to aerobic microorganisms; however, anaerobes rarely are implicated in clinically significant bacterial contamination of platelets.

Typically, samples from platelet units are obtained after an initial "lag phase" of at least 24 hours to allow growth of the initial contaminating inoculum. Units then may be released for distribution immediately after inoculation of samples into the growth detection system, or after a set pre-release incubation time. Using this approach to prevent product shortages introduces the likelihood that a small number of units issued for distribution and transfusion will subsequently prove positive for bacterial contamination. These events will occur for one of two reasons:
* The inoculation of samples may have introduced bacteria into the culture system that are not present in the distributed blood component (i.e., a "false positive" result-an event reported to occur in 1 in 500 to 1 in 5,000 inoculations, depending on technique and equipment used.)
* The contamination is real (a "true positive") but the bacteria are slow growing strains, or present in very small numbers, taking several days to achieve detectable titers. Preliminary data from those currently screening platelet concentrates suggests that true positives tend to show up earlier than false positives.

Algorithms for identifying bacterial growth in an already-transfused blood component are being developed by hospitals and blood centers. Presumably, this additional information about the organism and its antibiotic susceptibility will assist in managing the transfusion recipient if clinical signs of infection have developed.

Bacterial detection in whole blood derived platelets remains a challenge at this time. Because multiple

units must be sampled per transfusion dose, sampling techniques, component management logistics, and cost are barriers to culture-based assays. A number of non-culture based methods have been proposed to detect contamination prior to release: staining (Gram, Wright, or acridine orange) with microscopic examination,[4] chemistry assays (glucose, pH) using urine dipsticks,[7] and inspection for a peculiar light diffraction phenomenon called "swirling." These methods are limited in their sensitivity and specificity, but are acceptable alternatives to meet accreditation requirements (with the exception of "swirling," which is considered a secondary detection measure that requires trained and proficient staff).

Apheresis platelets may not be in sufficient supply or may not be the component of choice, so a gap between the safety profile of bacterially-screened apheresis platelets and bacterially unscreened whole blood derived platelets appears unavoidable until more sensitive methods applicable to platelets from whole blood are available. Alternative techniques are under development to bridge that gap.

SUMMARY

Bacterial contamination of platelets remains the most frequent infectious risk associated with transfusion. Improved phlebotomy techniques and diversion devices will help reduce the inoculation of skin flora into blood components. Detection of bacteria by culture-based assays of platelets collected by apheresis should allow interdiction of contaminated units prior to transfusion or, in some cases, early management of clinically significant complications arising from the infusion of bacteria during transfusion. Challenges for detecting contaminated whole blood derived platelets remain.[8] In the near term, quarantining for 24 hours prior to obtaining platelet aliquots for bacterial testing in practice reduces platelet shelf life to 4 days instead of 5. If proposed studies demonstrate that the techniques under discussion interdict bacterially contaminated platelets, platelet storage could be increased to 7 days. Taken as a whole, these steps add a margin of safety to current transfusion practices.

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