



Review paper

Bloodstream infections – etiology and current microbiological diagnostics

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ABSTRACT

Introduction: Bloodstream infections (BSI) and sepsis constitute an important clinical problem worldwide, with a high mortality rate. A fast and reliable determination of BSI etiology is necessary for administration of targeted antibiotic therapy and improvement in survival rates of patients with BSI.

Aim: To present the possibilities for optimal laboratory diagnostics of BSI, in the aspect of epidemiology, available research methods and current diagnostic recommendations.

Material and methods: A review of literature concerning recommendations in diagnosis and peer-reviewed publications using the following keywords ‘bloodstream infection,’ ‘bacteremia,’ ‘epidemiology,’ and ‘diagnostics.’

Results and discussion: Etiology of BSI depends on several factors, such as origin of microorganisms, the location of the primary source of infection, immunocompetence of the host, and possible contact of the patient with the healthcare. Blood culture has status of the ‘gold standard,’ which inables susceptibility testing of the isolated pathogen. Non-culture methods based on detection of microbial genetic material or proteins are increasingly used in laboratory diagnostics of BSI. They constitute the methods of choice in detection of uncultivable or difficult to culture microorganisms. New diagnostic solutions are urgently needed for rapid detection of multidrug-resistant strains of microorganisms.

Conclusions: Blood culture remains the reference method in laboratory diagnostics of BSI, while molecular techniques available at present are a valuable addition to it. However, the clinical relevance of the results of molecular tests which do not correlate with clinical symptoms needs to be solved.

1. INTRODUCTION

Bloodstream infections (BSI) constitute an important and still challenging medical problem worldwide. There are 575 000–677 000 episodes of BSI in USA and Canada, and over 1 200 000 episodes in Europe per year, out of which 79 000–94 000 and 157 000, respectively, are fatal.¹ Until 1980s mortality in sepsis and septic shock exceeded 80%, while at present it is about 30%–35%, as a result of improvement in treatment possibilities.² High death rates are recorded in cases of ineffective antibiotic therapy. It has been determined that every hour of delay in commencement of an antibiotic therapy effective against the pathogen causing sepsis decreases the survival rate by 7.6%.² The situation is complicated by the fact that clinical symptoms linked to the seeding of microorganisms to the bloodstream are not specific, therefore on this basis the etiology of BSI cannot be determined, particularly in patients with immune deficiency and/or subjected to intensive therapy.³ Therefore the importance of microbiological tests and the availability of rapid access to the identification and susceptibility testing, is being emphasized. Different microorganisms may be etiological agents of BSI – viruses, bacteria, fungi and parasites.⁴ Etiology of BSI to a large extent is dependent on the immunocompetence of the host, origin of microorganisms and the location of the primary source of infection.^{4,5} Profiles of microorganisms being detected in the blood of patients, are linked to the endemic or socioeconomic situation in specific institutions or geographic regions.^{6,7} Determination of BSI etiology is based on culture and non-culture methods. The diagnostic challenge is due to a broad profile of potential pathogens, usually a low load of microorganisms often intermittent presence of microorganisms in the bloodstream and a risk of unreliability of the result due to possibility of contamination. In the present study we describe etiology of BSI and methods of laboratory diagnosis of bacteraemia and fungaemia.

2. AIM

Determination of the possibilities for optimal laboratory diagnostics of BSI, including epidemiology, available research methods and current diagnostic recommendations.

3. MATERIAL AND METHODS

A review of the literature concerning recommendations in laboratory diagnostics and peer-reviewed publications using the following key words ‘bloodstream infection,’ ‘bacteraemia,’ ‘epidemiology,’ and ‘diagnostics.’

4. RESULTS AND DISCUSSION

4.1. Etiology of BSI

The frequency of occurrence of different microorganisms in the blood cultures to a large extent depends on the immu-

nological status of the patient (including the age and underlying diseases) and location of the primary infection, from which the microorganisms reach the bloodstream. Contact of the patient with the healthcare is also of importance.^{4,5} In children the most common etiological agents of BSI are *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Streptococcus aureus*, and *Escherichia coli*, while sepsis in neonates is most often caused by *Streptococcus agalactiae* and other β -haemolytic streptococci, *Enterobacteriales*, *S. aureus*, coagulase-negative staphylococci (CNS), *Listeria monocytogenes*, *Enterococcus* spp., *Pseudomonas* spp., and yeasts.^{4,8} In adults the most important etiological agents of bacteraemia acquired at hospital are: CNS, *E. coli*, *S. aureus*, Gram-negative rods of the order *Enterobacteriales* other than *E. coli*, as well as *Pseudomonas aeruginosa*, *Enterococcus* spp., anaerobes and fungi.^{4,5} In adults with community-acquired bacteraemia, the following pathogens are being isolated most often: *S. aureus*, *S. pneumoniae*, *E. coli* and other Gram-negative rods of the order *Enterobacteriales*, *N. meningitidis*, and β -haemolytic streptococci.⁴ Sepsis in pregnant women and those who have recently given birth (puerperas) is mainly caused by *E. coli*, *Streptococcus pyogenes*, *S. agalactiae* and *Staphylococcus* spp., Gram-negative rods other than *E. coli* and strict anaerobes. Polybacteraemia may also be diagnosed in these patients due to the presence of polyspecies microflora inhabiting the epithelium of the genital tract.⁹ BSI caused by multiple pathogens and fulminant clinical course of infections caused by encapsulated bacteria (*S. pneumoniae*, *Haemophilus influenzae*, *N. meningitidis*, *Campylobacter jejuni* and *Bordetella holmesii*) is diagnosed in patients after splenectomy.¹⁰ In patients suffering with leukaemia and lymphoproliferative disorders, the most common blood isolates comprise: CNS, *Enterococcus* spp., β -haemolytic streptococci, Gram-negative rods of the order *Enterobacteriales* (including *Salmonella* spp.), *P. aeruginosa* and other non-fermenting rods, *L. monocytogenes*, *Corynebacterium* spp., *Mycobacterium* spp. *Candida* spp., and other opportunistic microorganisms.⁴ Etiology of BSI depends on the location of the primary source of infection, as the profile of microorganisms isolated from the blood of patients with pneumonia differs from those with urosepsis. A separate issue constitutes the etiology of infective endocarditis (IE). Among the microorganisms which are detected with classical methods predominate oral streptococci, enterococci, staphylococci and bacteria of the HACEK group (*Haemophilus*, *Aggregatibacter*, *Cardiobacterium*, *Eikenella*, and *Kingella*), while among the microorganisms not detectable in the blood cultures the most common are: *Coxiella burnetii*, *Bartonella* spp., *Aspergillus* spp., *Mycoplasma pneumoniae*, *Brucella* spp. *Legionella pneumophila* and *Tropheryma whipplei*.¹²

4.2. Microbiological diagnostics of BSI

Microorganisms responsible for bacteraemia and fungaemia have often specific growth requirements regarding food substrates, as well as temperature, atmosphere and duration of incubation.⁴ Some pathogens, as mentioned earlier, cannot be cultured *in vitro*. For the above mentioned reasons, at

present there is no single diagnostic method which enables identification and characterisation of all etiological agents of BSI. However, when bacteraemia or fungaemia is suspected, in the routine diagnostic algorithm blood culture remains a reference method.^{12,13}

4.2.1. Microbiological testing of blood using a culture method – ‘blood culture’

Blood culture method remains ‘the gold standard’ amongst other techniques due to its versatility, relatively easy for implementation methodology and universality of its use in diagnostic laboratories all over the world. Indications for blood culture comprise a suspicion of bacteraemia, sepsis, IE, inflammation of the large blood vessels, fever of unknown origin, control of treatment efficacy (*S. aureus*, *E. faecalis*) and many other conditions. Due to automation of the laboratory phase of blood culture and the use of commercially available reagents, at present the biggest uncertainty is linked to the pre-analytical phase of the diagnostic procedure, which is of utmost importance in the laboratory diagnostics of BSI.^{12,13} Parameters, such as the volume and number of blood samples, as well as timing of their drawing, play a decisive role in obtaining a clinically relevant result. It is also important to adhere to the guidelines, such as drawing of the blood sample before the implementation of antibiotic therapy and proper disinfection of the skin at the site of venipuncture.^{4,12} The optimal time of sampling the blood for culture depends on the clinical status of the patient. In cases of severe illness with symptoms of sepsis, material should be obtained as soon as possible within 1 h.^{4,12} The recommended number of blood samples is a minimum of 2 sets of bottles, where a set is defined as 1–3 bottles for culture of blood samples, obtained from a single venipuncture.¹² However, the key parameter for laboratory diagnosis of BSI is drawing of the optimal volume of blood for culture. It results from the fact, that the number of microorganisms in 1 mL of blood is low, usually in the range of less than 1–10 cfu/mL. It has been estimated that the culture of 20 mL and 30 mL of blood instead of 10 mL results in an increase of percentage of positive samples by

38% and 62%, respectively. Therefore it is recommended to obtain from adults 20–30 mL of blood and placing it in 2–3 bottles with culture medium. The number of microorganisms in the blood is usually higher in neonates and children than in adults, and an optimal volume of tested blood depends on the age and body mass. Recommendations for an optimal sampling of the blood for culture are shown in Table 1.¹² Independently of age, the recommended material for microbiological testing is blood obtained directly from the peripheral blood vessel (usually from the vein), while taking blood samples from the vascular catheters is not recommended, except for laboratory diagnostics of catheter-related BSI (CRBSI).^{4,12} For skin disinfection before obtaining a blood sample for culture, the highest efficacy have the iodine tincture, chlorine peroxide, and chlorhexidine gluconate (not recommended for children less than 2 months of age).¹² Bottles inoculated with blood samples should be transported to the microbiology laboratory at room temperature as quickly as possible.^{4,12} The analytical stage of blood culture diagnostics is carried out in the laboratory, most often with the use of automatic systems dedicated to the detection of microorganisms in the blood.^{4,12} These systems consist basically of two elements cooperating with each other: culture media and apparatuses which ensure the maintenance of appropriate parameters of incubation, as well as detection and signaling of the microbial growth. The culture media contain the necessary nutrients for a wide range of microorganisms, as well as ensure the correct atmosphere for culture. They also contain anticoagulants, most often sodium polyanethole sulfonate (SPS). There are different types of blood culture bottles available – for aerobic or anaerobic microorganisms, mycotic bottles for a selective culture of fungi, lytic bottles containing agents which lyse the cellular components of the blood, or medium with antibiotic inhibitors.¹³ Special bottles and a unique test protocol are used for laboratory diagnostics of infections caused by bacteria of the genus *Mycobacterium*.¹² Blood culture systems detect the microorganisms by indirect methods with the use of measurement of the volume of CO₂ in the culture medium. The time recommended

Table 1. Optimal parameters of blood samples for laboratory diagnostics of BSI using blood culture method in relation to the patient’s age (Miller JM,¹² modified).

Patients: adults or children, body mass in kg	Volume of blood per culture set, ^a mL	Types of bottles in the blood culture set	Number of sets of blood culture bottles	Total blood volume for culture, mL
Adults	20 (30 ^b)	2 (3 ^b) bottles – at least 1 aerobic and 1 anaerobic	2–4 in each septic episode	40–80 (60–120 ^b)
Body mass <1.0 kg	2	1 bottle – aerobic	1	2
Body mass 1.1–2.0 kg	2	1 bottle – aerobic	2	4
Body mass 2.1–12.7 kg	4 ^c /2 ^d	1 bottle – aerobic	2	6
Body mass 12.8–36.3 kg	10	1 bottle – aerobic	2	20
Body mass >36.3 kg	20 (30 ^b)	2 (3 ^b) bottles – at least 1 aerobic and 1 anaerobic	2–4 in each septic episode	40–80 (60–120 ^b)

Comments: ^a blood culture set, consisting of all bottles inoculated with a blood sample drawn from a single venipuncture; ^b an additional 10 mL of blood is drawn in case of use of a special bottle for culturing fungi (mycotic bottle); ^c first set of blood culture bottles; ^d second set of blood culture bottles.

for this method is usually 5 days. The user has the option of prolongation of the incubation time of the samples. A 'positive' bottle should be replated on a set of appropriate culture media and direct preparation should be made and stained by a Gram method. The microorganisms detected in the blood culture samples should be identified to at least the genus level, however every effort must be made to obtain a full identification to the species.⁴ Blood culture bottles in which no microbial growth has been detected, automatically get a status of 'negative' bottles and there is no need to confirm this result with other diagnostic methods.

The method of blood culture has its limits, mainly a relatively long waiting time for a result (24 h – 5 days), false-negative results (low sensitivity) and false-positive results (e.g. contamination of the sample with microorganisms which originate from another source than blood). This phenomenon occurs with a variable frequency (mean 3%), and its most common cause are shortcomings in the procedure of skin disinfection.^{12,13} For this reason, BSI cannot be diagnosed solely on the basis of culturing CNS, *Micrococcus* spp., *Propionibacterium acnes*, *Bacillus* spp. or *Corynebacterium* spp. from only a single blood culture bottle.^{4,12} Falsely-negative results are those in which no microorganism has been detected in culture, despite an active infection in the cardiovascular system. Among the reasons of falsely-negative results of blood culture – other than an uncultivable etiological agent – are: an inappropriate moment of sampling (e.g. during intermittent bacteraemia, during so called 'temperature peak' or during antibiotic therapy), too low volume of the blood sample, insufficient number of obtained sets of blood samples, inappropriate storage and transport to the laboratory of blood culture bottles inoculated with the blood samples.^{4,12} It has been estimated that in critically ill patients the sensitivity of blood culture is about 70%, and the yield is lower in cases of fungaemia – even half of these cases may not be detected.¹²

4.2.2. Non-culture methods in laboratory diagnostics of BSI

There are two groups of commercially available tests which are increasingly used in laboratory diagnostics of bloodstream infections – based on detection of microbial genetic material or proteins. The use of these modern diagnostic methods for identification of microorganisms in positive blood cultures is one of the options of shortening the time needed for obtaining the result, in comparison to the classical method. Using these techniques, identification of a microorganism, and sometimes also information about the selected resistance mechanisms, may be available within about 1 to 6 h.^{13,14}

The genetic methods, are based on DNA amplification, with the use of polymerase chain reaction (PCR), which can be coupled with sequencing or microarray technology.^{12,13} In another group of genetic tests, fluorescent *in situ* hybridisation (FISH) is used, in which labelled probes bind specifically to the genetic material of the searched pathogen.

In the scope of proteomics, a recommended method is matrix-assisted laser desorption/ionization time-of-flight mass

spectrometry (MALDI-TOF MS), in which bacteria and fungi are being identified in 'real time' on the basis of obtained profiles of microbial proteins, which are compared with a spectra database of the system.^{12,13} A detailed description of the commercial tests, auxiliary to the blood culture methods, is beyond the scope of this publication, while advantages and disadvantages of molecular methods are discussed below.

For obvious reasons, direct tests detecting the microorganisms in the blood using technology other than blood culture, constitute the tests of choice in case of uncultivable microorganisms. These tests may also be highly useful in diagnostics of infections caused by fastidious, slowly growing microorganisms (e.g. *Mycobacterium* spp.) or difficult to detect in blood culture (e.g. intracellular bacteria, fungi). Comparative testing of PCR assays of new generation with the standard technique (blood culture) point to the higher sensitivity of the genetic methods.¹³ Moreover, the result is obtained in a much shorter period of time than in culture and is not influenced by antibiotic therapy.¹⁵ Furthermore, in contrast to the blood culture, which is a qualitative technique, molecular techniques also allow quantitative evaluation of the sample. However, the range of commercial assays for direct testing of blood samples is limited to a few PCR tests belonging to the multiplex group or broad-range assays. The material for testing is a sample of 1–5 mL of full blood taken into the EDTA tube. In the first stage of the assay, DNA is extracted from the blood, most often in the automatic process. Subsequent stages are specific for a particular technology used in the test. Amplification process during the test may be 'aided' with the use of specific probes, electrophoresis, or microarray. For identification of the microorganisms – sequencing, mass spectrometry (PCR/ESI-MS) or melting curve analysis (real-time PCR) may be used.^{13,15} Multiplex panels enable detection of 20–34 different pathogens and single resistance genes (mainly *mecA*), and the result may be available within one to several hours. Systems from the group of broad-range PCR have much wider possibilities of microorganism identification, even more than 700 species, and some of them enable identification of dangerous resistance genes (*mecA*, *vanA*, *vanB*, *bla_{KPC}*). The time needed for getting a result of a broad-spectrum test ranges 6–12 h. However, tests for direct detection of pathogens in the blood have some limitations, such as lack of versatility of these assays and the possibility of identification of only a limited number of microbial species. With the use of one of the commercial platforms, it is possible to detect 19 specific species of bacteria, 5 species of yeasts and 3 resistance genes.¹⁵ There is also a possibility of inhibition of a PCR reaction by factors present in the full blood and a small number of microorganisms in the sample volume. In both cases it may lead to falsely-negative results. To avoid false-positive results due to contamination of the sample skin disinfection must be even more stringent when the specimen will be used for molecular testing. At the same time, at present the issue is not solved of what the clinical relevance of the results of molecular tests is, which do not correlate fully with clinical symptoms. The problem concerns the 'oversensitivity' of the method based on the detection of non-viable microorganisms, but only their genetic material,

which may be a physiological phenomenon with no clinical consequences. Similar doubts apply to the interpretation of the results of the detection of the resistance genes, due to the issue of their expression and the role of different mechanisms of resistance to antimicrobials in the determination of the resistance phenotype. For this reason at present there is an obligatory principle of susceptibility testing of the microorganism isolated in blood culture, and therefore a need for carrying on BSI diagnostics with the use of blood culture. For this reason optional testing with the use of advanced technologies contributes to an additional cost of hospitalisation, which can be justified (at present) in the diagnostics and therapy of patients in a severe clinical condition.

5. CONCLUSIONS

- (1) Blood culture remains the reference method in diagnostics of BSI.
- (2) Molecular techniques available at present do not constitute an alternative as yet, however they are a valuable addition to the reference method.
- (3) There is an urgent need for improvement and elaboration of new diagnostic solutions in view of the threat posed by sepsis and systemic infections caused by multi-drug-resistant strains of microorganisms.

Conflict of interest

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