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Continuing medical education: Methods of rapid diagnosis

Molecular methods for septicemia diagnosis☆

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ABSTRACT

Septicemia remains a major cause of hospital mortality. Blood culture remains the best approach to identify the etiological microorganisms when a bloodstream infection is suspected but it takes long time because it relies on bacterial or fungal growth. The introduction in clinical microbiology laboratories of the matrix-assisted laser desorption ionisation time-of-flight mass spectrometry technology, DNA hybridisation, microarrays or rapid PCR-based test significantly reduce the time to results. Tests for direct detection in whole blood samples are highly desirable because of their potential to identify bloodstream pathogens without waiting for blood cultures to become positive. Nonetheless, limitations of current molecular diagnostic methods are substantial. This article reviews these new molecular approaches (LightCycler SeptiFast, Magicplex sepsis real time, Septitest, VYOO, PCR/ESI-MS analysis, T2Candida).

Métodos moleculares para el diagnóstico de septicemia

Palabras clave:
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RESUMEN

La septicemia es una de las causas más importantes de muerte en pacientes hospitalizados. El hemocultivo es el método de referencia para detectar el agente etiológico responsable, pero el resultado definitivo depende de la velocidad de crecimiento del microorganismo. En los últimos años el empleo de diversas tecnologías como la espectrometría de masas *(matrix-assisted laser desoption ionization time-of-flight)*, la hibridación del ADN, los *microarrays* o las reacciones de PCR rápidas han disminuido de forma considerable el tiempo necesario para la identificación de los microorganismos y la detección de genes de resistencia a partir de hemocultivos positivos. El diagnóstico molecular de una septicemia directamente de la sangre del paciente permite conocer el resultado en pocas horas, aunque todavía existen diversas limitaciones que dificultan su empleo. En esta revisión se exponen los diversos métodos moleculares disponibles (LightCycler SeptiFast, Magicplex sepsis real time, Septitest, VYOO, PCR/ESI-MS análisis, T2Candida) y su posible utilidad.

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Introduction

Early microbiological diagnosis of a circulatory system infection caused by bacteria (bacteraemia), fungus (fungaemia) or virus (viraemia) should be a priority objective of any microbiology laboratory. In severe clinical symptoms that evolve towards a serious situation of septicaemia with shock, adoption of appropriate therapeutic measures and administration of proper antimicrobial treatment as early as possible are essential for decreasing the high morbidity and mortality observed in these cases.[1] Septicaemia is one of the leading causes of death in hospitalised patients. According to the data collected in various publications, in which European and North American patients are included, the number of deaths attributable to this clinical condition is estimated at 400,000 per year.[2–4] From a clinical perspective, septicaemia presents as an imprecise syndrome for which the diagnosis is based on the clinical suspicion of an infection combined with signs of organ dysfunction. Confirmation of septicaemia requires identification of the aetiological agent. To date, the standard recommended methodology is based on performing haemocultures that, if positive and using traditional methods, require a minimum of 48–72 h to obtain the result identifying the microorganism responsible and its susceptibility to antibiotics. The yield of haemocultures is variable. If 2–4 haemocultures are collected (40–80 ml of blood) before starting antimicrobial treatment, the aetiological agent is detected in 80–96% of cases.[5,6] However, haemocultures are negative in a high proportion of cases (50%) when the patient has severe septicaemia.[7] This may be due to various factors, such as prior antimicrobial treatment, few microorganisms circulating in the blood or non-culturable or slow-growing microorganisms. Also, it is estimated that in patients with septic shock, each hour that elapses from the start of hypotension to the administration of active antibiotics causes a mean decrease in survival of 7.6%.[8] Since antimicrobial treatment is a critical factor in the survival of patients with septicaemia, broad-spectrum antibiotics are usually used initially to cover all possible pathogenic agents and later, based on the results of the haemocultures, the treatment is adapted. Therefore, it is clear that our goal should be to shorten the time needed to reach a microbiological diagnosis of septicaemia. In light of this challenge, two options are posited. The first one — which is, in theory, the most desirable due to the immediacy of the diagnosis — involves identifying the microorganism responsible for the septicaemia directly from the patient's blood. The second option seeks to identify the aetiological agent as soon as possible once the haemoculture has been found to be positive. In both cases, detection of resistance genes to the most common antibiotics and/or determining susceptibility to antibiotics should also be possible.

Direct diagnosis from blood

The application of molecular techniques directly on whole blood samples offers the possibility of identifying the aetiological agent responsible for the septicaemia in a short period of time. Also, depending on the method used, it is possible to detect the presence of certain antibiotic resistance genes, facilitating the choice of the most appropriate antimicrobial treatment. This diagnostic option has benefited in recent years from the changes made to techniques that allow for extraction of nucleic acids, their amplification methods and the possibility of using multiplex polymerase chain reactions (PCRs) to increase diagnostic options. However, and a priori, use of these molecular techniques must face various disadvantages. There is a large amount of human DNA in the patient's blood, along with contaminating DNA and persistent DNA from dead microorganisms. The presence of PCR inhibitors, such as iron ions or immunoglobulins, must also be kept in mind.[9] The amount of DNA present can be reduced by extracting leukocytes or using methods that allow it to be extracted or degraded specifically. Also, anticoagulants such as heparin should be avoided due to the risk of PCR inhibition, and EDTA will be used. The second disadvantage that should be kept in mind is the low number of microorganisms circulating in the blood during an episode of bacteraemia, which is estimated between 1 and 10 CFU/ml.[10] These values are based on quantitative studies conducted with conventional methods that perhaps do not represent the real number of circulating and viable microorganisms. Bacconi et al.[11] suggest that for methods like PCR, it is better to consider the number of genomic copies (GC) of a microorganism present in a sample. This concept would also consider the DNA of dead bacteria or those captured by circulating phagocytic cells. According to this option, it is estimated that in an episode of bacteraemia the number of circulating GCs would be between 10^3 and 10^4/ml. This value would be higher than the detection limit for most PCRs.

Various systems that can be used in direct diagnosis from whole blood have been marketed. The ideal technique should consider the following features: speed, high sensitivity and specificity, capacity to detect non-culturable microorganisms, detection of various resistance mechanisms, the highest automation possible, easy to implement in the daily routine of a microbiology laboratory and being cost-effective. It is difficult to meet all these requirements and it should also be taken into account that using this technique has certain disadvantages, such as not having the identified microorganism or contamination of the process with external genetic materials, which could hamper interpretation. All studies conducted to date with these new molecular methods raise certain questions about their actual sensitivity, since they are compared to the standard haemoculture, which is probably not sufficiently sensitive to be considered a reference method in the diagnosis of septicaemia. For the reasons commented, the most sensible option would be to use both methods and evaluate the results based on the patient's clinical situation.

LightCycler SeptiFast

The LightCycler SeptiFast system (Roche Molecular System, Switzerland) was the first to be marketed and has been evaluated in various clinical studies.[12–16] It allows for direct detection and identification from blood of 25 pathogens that make up 90% of the most common aetiological agents in septicaemia. The study panel includes the gram-negative bacilli: *Escherichia coli*, *Klebsiella (pneumoniae/oxytoca)*, *Serratia marcescens*, *Enterobacter (cloacae/aerogenes)*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*; gram-positive cocci: *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptococcus pneumoniae*, other strains of*Streptococcus*, *Enterococcus faecalis*, *E. faecium*, and *Candida* spp. (5 species) and *Aspergillus fumigatus*. It can also detect the presence of the *mecA* gene, responsible for methicillin resistance. It only requires 1.5 ml of whole blood, and the duration of the detection process is 3.5–6 h, depending on the results. The system uses the sequences located between 16S and 23S of ribosomal DNA as the target for identifying the bacteria, and, for fungi, those located between 18S and 5.8S. Once the DNA has been extracted, it is purified and 3 multiplex PCRs are conducted (gram-negative bacteria, gram-positive bacteria and fungi) which allows for identification of the microorganisms included in the panel on a genus and species level, based on the analysis of the melting points of the amplicons obtained. The method's detection limit is 3–30 CFU/ml for bacteria and 100 CFU/ml for yeasts.

Results of several clinical studies conducted on patients with severe septicaemia,[13,14] neutropaenic patients with fever,[13,15] paediatric patients[16] or patients admitted to intensive care units[13]