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Review article

Microbiological diagnosis of biofilm-related infections☆

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ABSTRACT

Biofilm-related infections represent a serious health problem, accounting for 65–80% of all infections. The infections are generally chronic and characterised by the persistence of the microorganism, due to the increased resistance of biofilms to both the immune system and antimicrobials. Biofilms can be located to almost every human body tissue and on exogenous devices such as catheters, pacemakers, prosthetic material, implants, urinary catheters, etc.

Traditional antimicrobial susceptibility studies in clinical microbiology laboratories have lied on the study of planktonic form of microorganisms. However, this approach might lead to miss the biofilm characteristics and to a treatment failure. Microbiological diagnosis and antimicrobial susceptibility studies of biofilm-related infections are complex and, nowadays, represent a challenge that clinicians and microbiologists have to address as a team in the absence of consensus or standardised protocols.

Diagnóstico microbiológico de las infecciones relacionadas con la formación de biopelículas

Palabras clave:
Biopelículas
Sensibilidad antibiótica en biopelículas
Diagnóstico microbiológico en infecciones por biopelículas

RESUMEN

Las infecciones asociadas a biopelículas suponen un grave problema sanitario ya que representan entre el 65 y el 80% de todas las infecciones. Estas son generalmente crónicas y están caracterizadas por la persistencia del microorganismo debido a su resistencia al sistema inmunitario y a los antimicrobianos. Las biopelículas se pueden localizar tanto en tejidos humanos como sobre dispositivos exógenos tales como catéteres, marcapasos, prótesis, implantes, sondas urinarias, etc.

Tradicionalmente, los laboratorios de microbiología clínica realizan los estudios de sensibilidad sobre microorganismos en crecimiento planctónico. Sin embargo, de esta manera se pierden las características propias de la biopelícula con lo que la antibioterapia basada en estos estudios podría asociarse con fracaso terapéutico o recurrencias. El diagnóstico microbiológico y los estudios de sensibilidad en las infecciones relacionadas con biopelículas son complejos y, hoy por hoy, representan un reto que clínicos y microbiólogos han de abordar en equipo ya que no existe todavía un consenso global ni protocolos estandarizados.

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Introduction

Biofilms are structured supracellular formations which develop as a survival strategy in hostile environments, supplying the microorganisms embedded in them with resistance to mechanical clearance, the immune system and to antimicrobial agents.[1,2]

Biofilm-related infections, which are typically persistent chronic infections refractory to antimicrobial treatment, represent a significant health problem as they account for 65–80% of all infections. These can be located on almost any tissue of the human body, including chronic skin and soft tissue infections, lung infections in patients with cystic fibrosis (CF) or bronchiectasis, or endocarditis.[2] Biofilms also cause infections related to various biomedical devices. In general, these infections are difficult to diagnose and treat. There is currently much uncertainty with regard to the optimal therapeutic strategy for these patients.[3] Traditionally, clinical microbiology laboratories have focussed on isolating and conducting susceptibility studies on microorganisms in planktonic state. However, releasing microorganisms from biofilms means that the biofilms lose their characteristics, and this can lead to mistakes in the extrapolation of data on antimicrobial susceptibility in planktonic state. This paper addresses both the microbiological diagnosis and susceptibility studies in biofilm-related infections.

Infections associated with the formation of biofilms on tissues and devices

Chronic lung infection

Chronic lung infections such as CF, chronic obstructive pulmonary disease and bronchiectasis represent a predisposing factor for chronic infection. The most prevalent microorganisms in this context are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[4–8] Suitable samples for microbiological diagnosis are spontaneous or induced sputum, bronchoalveolar lavage (BAL) or bronchial suction, trying to minimise oropharyngeal contamination while collecting the samples.

Chronic rhinosinusitis

This is an inflammatory condition which affects the mucous membranes of the paranasal sinuses and the nasal passages. It tends to start with a viral infection which, in some cases, progresses and develops into a secondary bacterial superinfection frequently caused by *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*. If the viral infection is not resolved, colonisation by anaerobic oropharyngeal microbiota (such as *Fusobacterium nucleatum*, *Prevotella* spp., *Porphyromonas* spp. and *Peptostreptococcus* spp.) and aerobic oropharyngeal microbiota (*P. aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter* spp. and *Escherichia coli*) and *S. aureus* (including methicillin-resistant *S. aureus* [MRSA]),[9] even fungi such as *Aspergillus* spp., generally in elderly and/or immunocompromised patients, predominates. Purulent secretions obtained from the middle meatus or through the cavities of the paranasal sinuses are the preferred samples for microbiological diagnosis.

Chronic otitis media

Otitis media is an infection, which can be acute or chronic, with the presence of exudate in the middle cavity of the ear. The bacteria commonly involved are *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*.[10] *P. aeruginosa* and *S. aureus* are the most commonly involved microorganisms in chronic suppurative otitis media and chronic otitis media with cholesteatoma. Microbiological diagnosis is generally reserved for cases which are refractory to treatment. The clinical sample should be obtained by means of aspiration through tympanocentesis, and, if there is spontaneous tympanic perforation, the exudate which flows to the external canal of the middle ear can be used. This sample will be taken with a syringe whenever possible, or, failing that, with a swab.

Chronic wound infection

A chronic wound is considered to be that in which the healing process does not take a normal course and the functional and anatomical integrity of the skin is not achieved after approximately one month. Infection is the main cause of this chronicity.[2,11] All open wounds are colonised by endogenous and exogenous microorganisms, although biofilms are usually composed of only one bacterial species, basically *P. aeruginosa* and *S. aureus*. Anaerobic microorganisms may also be involved (*Bacteroides* spp., *Prevotella* spp., *Porphyromonas* spp. and *Peptostreptococcus* spp.), *Bacillus anthracis*, beta-haemolytic streptococci, *Enterococcus* spp. and Enterobacteriaceae. A culture from the deep tissue biopsy is recommended for the microbiological diagnosis.

Infection in burn patients

Although the surface of the burns is initially sterile, microbial colonisation occurs rapidly: Gram-positive bacteria colonise the wound in the first 48 h, and, after 5–7 days, it may be colonised by other Gram-positive and Gram-negative bacteria, and later on by yeast from normal microbiota. The majority are monomicrobial infections and the most common microorganisms are *P. aeruginosa* and *S. aureus*. Other less common microorganisms are *Acinetobacter baumannii*, *Enterococcus faecalis* (*E. faecalis*), *E. coli*, *K. pneumoniae* and *Enterobacter* spp. The diagnosis is arrived at by clinical suspicion and quantitative culture (10^5 CFU per gram of tissue) of the biopsy material.

Native heart valve infection

Native valve endocarditis is caused by an interaction between the vascular endothelium and circulating microorganisms in the blood which multiply in the lesion forming a biofilm in the form of vegetations. The vegetations can prevent the correct functioning of the valve, generating a continuous source of microorganisms to the bloodstream and a risk of remote septic embolisms. This condition continues to have a high mortality rate and its main causative agents are *S. aureus* (31%), *viridans* group streptococci (17%), *Enterococcus* spp. (11%), coagulase-negative staphylococci (11%), *Streptococcus bovis – S. bovis* (7%), other streptococci (5%), Gram-negative bacilli (2%), fungi (2%), Gram-negative bacilli of the HACEK group (2%, *Haemophilus aphrophilus* [*Aggregatibacter aphrophilus*, *Aggregatibacter paraphrophilus*], *Actinobacillus actinomycetemcomitans* [*Aggregatibacter actinomycetemcomitans*], *Cardiobacterium hominis*; *Eikenella corrodens* and *Kingella kingae*[12]). The diagnosis is based mainly on the positivity of the blood cultures, which should be incubated for more than five days if endocarditis is suspected, and on the echocardiography. Nevertheless, the blood cultures may turn out to be negative (5–30% of cases) due to concomitant antibiotic therapy or in endocarditis caused by fungi or by fastidious microorganisms. Therefore, molecular techniques and serology may be useful. A positive culture from the vegetation is considered a major criterion for the diagnosis of endocarditis. A valve culture is not useful.