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Comparison of Genus Specific PCR and Culture with or without Sonication for Microbiological Diagnosis of Vascular Graft Infection☆

Mathilde Puges [a,*], Sabine Pereyre [b,c], Xavier Bérard [d], Isabelle Accoceberry [e], Chloé Le Roy [b], Laurent Stecken [f], Jean-Baptiste Pinaquy [g], Arnaud Desclaux [a], Michel Dupon [a], Cécile Bébéar [b,c], Charles Cazanave [a,b]

[a] Infectious and Tropical Diseases Department, University Hospital of Bordeaux, Bordeaux, France
[b] USC EA 3671 Mycoplasmal and Chlamydial Infections in Humans, University Hospital of Bordeaux, French National Reference Centre for Bacterial STIs, Bordeaux, France
[c] Bacteriology Department, University Hospital of Bordeaux, Bordeaux, France
[d] Vascular Surgery Department, University Hospital of Bordeaux, Bordeaux, France
[e] Mycology and Parasitology Department, University Hospital of Bordeaux, Bordeaux, France
[f] Anaesthetics Department, University Hospital of Bordeaux, Bordeaux, France
[g] Nuclear Medicine Department, University Hospital of Bordeaux, Bordeaux, France

WHAT THIS PAPER ADDS

Conventional microbiological methods lack sensitivity in VGI. By combining sonicate fluid culture and PCR, a microbiological diagnosis was obtained for all patients with VGI in the study. However, there was no statistical difference between performances of culture with and without sonication and genus specific PCR. This suggests that combining sonicate fluid cultures and PCR may be the best strategy for microbiological diagnosis of VGI. Moreover, the study highlighted the high frequency of fungal infections in aorto-enteric fistulas.

Objectives: Vascular graft infections (VGIs) are severe and require prolonged adequate antimicrobial therapy. However, up to 45% of conventional cultures are negative. Sonication and genus specific PCRs for microbiological diagnosis of VGI was evaluated.

Methods: Samples were prospectively obtained from explanted vascular grafts in Bordeaux University Hospital. Conventional bacterial cultures with and without prior sonication of samples were performed. A genus specific PCR assay panel, targeting the most frequent bacteria involved in VGI (*Staphylococcus*, *Streptococcus*, *Enterococcus*, and Enterobacteriaceae), was also applied to sonicate fluids. The performance of these three diagnostic strategies was compared.

Results: Forty-five patients (118 samples) were included between July 2014 and October 2015. Six patients had no infection and 39 had a VGI. Sensitivities of graft culture, sonicate fluid culture, and genus specific PCR were 85.7%, 89.7%, and 79.5%, respectively. Specificities were 100%, 100%, and 83.3%, respectively. Sonicate fluid culture was positive for five graft samples (from four patients) with negative culture without sonication. Four VGIs were detected by PCR only (3 patients had previously received antibiotics). For 15 patients with positive graft cultures, PCR identified at least one additional bacterium compared with culture, thus 30 additional bacteria for all included patients. By combining sonicate fluid culture and PCR, a microbiological diagnosis was obtained for all patients with VGI.

Conclusions: There was no statistical difference between performances of culture with and without sonication and genus specific PCR. However, combining sonicate fluid cultures and PCR may be the best strategy for microbiological diagnostic of VGI.

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INTRODUCTION

VGI is associated with high morbidity and high mortality rates,[1,2] increased in aortic VGI[3] especially with aorto-enteric fistula.[4] When possible, optimal management of these infections requires total surgical excision of the infected graft and long-term antimicrobial treatment.[1,5] An early microbiological diagnosis is therefore necessary to direct appropriate antimicrobial treatment. However, conventional microbiological methods lack sensitivity, as 4–45% of VGIs remain culture negative,[1] notably 30% in the

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* Corresponding author. Infectious and Tropical Diseases Department, University Hospital of Bordeaux, Place Amelie Raba Leon, 33076 Bordeaux, France.

E-mail address: mathilde.puges@chu-bordeaux.fr (Mathilde Puges).

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authors' hospital.[6] This could be explained both by frequent use of antimicrobial therapy before graft removal, and by the presence of biofilm associated microorganisms.[7] These issues are similar to those encountered in prosthetic joint infection[8,9] and cardiac material related infection,[10,11] where new strategies have been studied to improve microbiological diagnosis, especially sonication coupled to molecular biology. Sonication and broad range polymerase chain reactions (PCRs) have already been evaluated in VGI in one recently published study.[12] However, there was no comparison with conventional culture. Moreover, genus specific PCRs have never been evaluated in VGI diagnosis.

A prospective study was conducted to compare the performances of conventional culture without sonication, of sonicate fluid culture, and of genus specific PCR applied on sonicate fluids for microbiological diagnosis of VGI.

METHODS

Study population

Vascular grafts explanted for suspected VGI or other non-infectious reasons (graft rupture, anastomosis rupture) were prospectively and systematically collected from July 2014 to October 2015 at Bordeaux University Hospital, Bordeaux, France. All types of vascular grafts were included, biological grafts (arterial or venous allo/homograft) and synthetic grafts (polyester or polytetrafluoroethylene vascular grafts, endografts and stents). Clinical signs, demographic and biological data were recorded for each subject. The study was approved by local ethics committee (Comité de Protection des Personnes Sud-Ouest et Outre Mer, reference DC 2014/144).

Definition of VGI cases

VGI was diagnosed according to Fitzgerald's criteria,[7] as previously reported[3] and detailed in Supplementary material 1. If those criteria were missing, diagnosis of VGI was rejected. Early onset VGIs, occurring within 4 months of prosthetic implantation, were distinguished from late onset infections, occurring more than 4 months after implantation.

Specimen collection

For each patient, several prosthesis samples were obtained and, sometimes, several grafts were explanted from a single patient. All intra-operative samples were processed at the laboratory within 2 hours. Each sample was divided into three parts, for sonication, conventional bacterial, and fungal cultures. The remaining sonicate fluid was used to perform specific real time PCRs panel assays. Other types of samples (blood cultures, pre-operative and per-operative samples) were processed according to standard laboratory procedures.

Conventional bacterial culture

Ten millilitres of brain—heart (BH) infusion broth were added to the first part of the samples and plated onto aerobic horse blood, polyvitex chocolate, and anaerobic Schaedler agars. Agar plates were incubated at 37°C for 48 hours in aerobic atmosphere with 5% CO_2, for 7 days in aerobic atmosphere with 5% CO_2, and for 10 days in anaerobic atmosphere. One millilitre of BH infusion broth was also inoculated into 10 mL of Schaedler broth. Schaedler broth and the remaining 9 mL of BH infusion broth were incubated at 37°C for 15 days. Cloudy broth media were subsequently plated on polyvitex chocolate agar plates and incubated in a 5% CO_2 atmosphere for 7 days. Isolated bacteria were identified according to standard laboratory procedures, using Matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany).

Conventional fungal culture

The second part of the samples was immersed in sterile saline isotonic fluid and vortexed for 30 seconds. Fluid was collected, centrifuged, and the pellet was inoculated onto a BBL CHROMagar Candida Medium (Becton Dickinson, Franklin Lakes, NJ, USA) and a Sabouraud Chloramphenicol Gentamicin agar (Bio-Rad, Hercules, CA, USA), and incubated aerobically at 37°C for 7 days and at 30°C for 21 days, respectively. Isolated fungi were identified according to standard laboratory procedures, using MALDI-TOF MS.

Sonicate fluid culture

The sonication process uses ultrasound applied to the graft to dislodge adherent microorganisms from explanted grafts. In the study, sterile saline isotonic fluid was added to immerse the sample, which was sonicated according to the protocol described for prosthetic joint infection.[9] Briefly, immersed grafts were vortexed for 30 seconds, sonicated for five minutes at a frequency of 51 ± 3 kHz and power density of 0.2 W/cm^2 (Bransonic 3200, Branson Ultrasonic, Slough, UK) and vortexed for another 30 seconds. Sonicate fluid culture was performed as described for conventional bacterial and fungal cultures. Two 1.5 mL aliquots of sonicate fluid were stored at −80°C. During the study, saline fluid from the sonication bath was regularly inoculated to detect any bacterial bath contamination. All sonication bath cultures remained negative during the study.

DNA extraction and genus specific PCR assay panel

A 1000 µL aliquot of sonicate fluid was used for DNA extraction, using NucleoSpin tissue kit (Macherey—Nagel, Hoerdt, France) according to the manufacturer's instructions and eluted in a volume of 50 µL. Before extraction, 3 µL of an internal control "DNA Extraction and Inhibition Control" (Dia-EIC/DNA) (Diagenode, Liège, Belgium) was added to each aliquot of sonicate fluid. The ion chromatography (IC) detection was performed separately by real time PCR using user defined workflow (UDF) software on a cobas z 480 analyser (Roche Diagnostics, Meylan, France) according to the procedures recommended by Diagenode. Results were validated if the cycle threshold (Ct) was between 27 and 35.

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