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Extended Abstract

Classical Microbiological Diagnostics Of Bacteraemia - Are The Negative Results Really Negative? What is Laboratory Result telling us About "Gold Standard"?

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Keywords:

Sepsis; bacteraemia; blood culture; Gram staining; Fluorescence in-situ Hybridization.

Abstract:

Standard blood cultures require at least 24-120 h to be reported as preliminary positive. The objective of this study was to match the equivalent of Gram staining and fluorescent in-situ hybridization (FISH) for bacteria in otherwise negative blood culture bottles. Ninety-six sets were arrested from patients with a analysis of sepsis. Six incomplete blood culture sets and eight blood cultures sets professed positive growth were refuse. We achieve Gram's Method and FISH on 82 sets taken from check septic patients: 82 negative aerobic blood cultures, 82 anaerobic blood cultures, and 82 blood samples, as well as 57 blood samples taken from active volunteers. Against eighty-two blood agreed analysed from the septic patients, Gram's Method visualized bacteria in 62.2% of blood samples, 35.4% of the negative aerobic bottles, and in 31.7% of the negative anaerobic bottles. Apply FISH; we detected bacteria in 75.6%, 56.1%, and 64.6% commonly. Among the blood samples from healthy volunteers, FISH detected bacteria in 64.9%, while Gram's Method detected bacteria in just 38.6%. The time needed to get the study results using Gram's Method was 1 h, for FISH 4 h, and for the culture method, considering the duration of growth, 5 days. Gram stain and FISH allow quick detection of bacteria within the blood taken directly from a patient. Discovery phagocytized bacteria, which were also catch among healthy individuals, confirms the hypothesis that blood micro biome exists.

1. Introduction

Sepsis is "a life-threatening organ dysfunction caused by a deregulated host response the infection" as defined by the Society of Critical Care Medicine and European Society of medical care Medicine Task Force (SCCM/ESICM Sepsis Definitions Task Force). The preliminary diagnosis is usually based on the clinical picture and is often made before any laboratory results become available [1], while the diagnostic and prognostic assessments are based on sepsis-related organ failure assessment (SOFA) or the quick SOFA (qSOFA) scores [2]. The SOFA score includes: PaO2/FiO2, the need of mechanical ventilation, Glasgow Coma Scale, mean arterial pressure, platelet count, bilirubin and creatinine level. The global incidence of sepsis is difficult to ascertain due to lack of or insufficient reporting, butit ranges from 73.6/100,000 in the United States in 1979 to 1180/100,000 in Northern Australia from2007 to 2008 [3,4]. In 2016, the global incidence of sepsis was estimated at 31 million cases per year (437 per 100,000) and its mortality, around 5 million/year. Being that sepsis is the main cause of mortality worldwide [5], the prompt identification of the source of infection and determination of the etiologic agent are important for optimization of therapy, including the choice of an efficient ,targeted antibiotic therapy, which can contribute to improved prognosis in patients with sepsis.

2. Materials and Methods

2.1. Patients

The study was conducted at the John Paul II Specialist Hospital in Cracow, Poland, which admits over 20,000 patients/year, and performs about 2000 open heart procedures and 1100 thoracic procedures annually. Blood samples (2 mL) and standard blood culture were taken from patients admitted to the Intensive Care Unit (ICU) (Table 1). The study was conducted from August 1, 2017 to December 31, 2018. The patients who took part in the study had undergone thoracic surgery subsequently developing post-operative pulmonary complications for which intravenous antibiotics had been initiated. During the course of the ICU stay, the patients demonstrated new signs and symptoms of sepsis (SOFA $\geq$ 2); laboratory tests were ordered including blood cultures and other basic labs. In total, 96 septic patients with a SOFA score $\geq$ 2 points were enrolled in the study. Exclusion criteria were SOFA score < 2, incomplete blood sets, and growth of bacteria in blood cultures. The 57 healthy volunteers were recruited by advertisements posted in the hospital's Bulletin Board. Inclusion criteria were the absence of inflammation markers (CRP, leucocytosis).

2.2. Samples

In total, 96 blood set were taken from septic patients with a SOFA score ≥ 2 points [2]: 2–3 blood sets were taken from different anatomical sites for normal microbiological diagnostics. A single set consisted of blood taken for aerobic and anaerobic bacterial cultures and a separate 2 mL blood sample. Under strict sterile conditions, blood cultures (aerobic and anaerobic bottles-FAN Plus media (bioMérieux) with adsorbent polymeric beads (APB) to neutralize antimicrobials) during the same phlebotomy, a 2-mL blood sample was also collected from the septic patients into VACUETTE® TUBE K3E (Becton Dickinson, Eysins, Vaud. Switzerland).

Statistical Analysis

The results were subjected to statistical analysis using parametric and nonparametric tests using IBM SPSS Statistics Software, ver. 25 (IBM Corp., NY, and 10504-1722 USA). Categorical variables were examined using the chi-square test or Fisher's exact test. Continuous variables were analysed using the t-test. Statistical hypotheses were verified at the significance level of $p < 0.05$.

3. Results

The patients who took part in the study met the SOFA criteria of sepsis after a thoracic surgery.

The mean SOFA score ranged from 6.6 in patients with positive blood cultures to 7.9 in patients with

Negative blood cultures. From the 96 sets taken from patients with a diagnosis of sepsis, six incomplete blood culture sets and eight blood culture sets demonstrating positive growth were excluded. The following

Microorganisms were found in blood samples excluded from the study: Staphylococcus aureus, S.epidermidis (two patients), Klebsiella pneumoniae, Streptococcus mitis, S. haemolyticus, Lactobacillus spp., and Candida albicans.

4. Discussion

Experts agree that sepsis defines a collection of organ failure symptoms caused by infection. Sepsis is a set of symptoms caused by systemic inflammation due to bacterial or fungal infection. Symptomatic bacteraemia does not necessarily mean sepsis, but it is classified as a blood stream infection (BSI) and requires microbiological diagnostic of blood.

5. Conclusions

FISH demonstrated greater sensitivity as compared with Gram staining. Both methods enable the detection of bacteria in media culture that did not show growth. Both methods revealed the presence of bacteria in the blood of patients with sepsis and the control group, which confirm there, ports to date regarding the constant 'physiological bacteraemia'.

Biography

Tomasz Zrodlowski graduated from Pomeranian Medical University in Poland, has completed his training in Anaesthesiology and Intensive Care in Poland and France, and currently is doing his Internal Medicine Residency in the United States. He is also a PhD student at the Jagiellonian Medical University in Poland.