Centre Hospitalier Universitaire de Liège

The microbial diagnosis of infective endocarditis (IE)

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Medical Microbiology

- Introduction
- Duke criteria for diagnosis
- Review of microbiological investigation of IE
- Key points
- Conclusion and perspectives

IE - Introduction

- **High morbidity and mortality**
  - **No decrease of incidence**
    - **Progressive changes of risk factors**
      - **↘ Classic risk factors**
      - **↗ New risks**
        - **IV drug use**
        - **Selective valve in elderly patients**
        - **Use of prosthetic valves**
        - **Nosocomial diseases**
        - **Haemodialysis patients**
      - **Newly identified pathogens difficult to cultivate**

IE - Introduction

- **Evolution of antimicrobial resistance**
  - **Multidrug R Streptococcus viridans**
  - **MRSA and CA-MRSA**
  - **VISA**
  - **VRE**
  - **Aminoglycoside high level R Enterococci**

- **Challenge for a successful outcome :**

  Diagnosis and subsequent treatment

Diagnosis of IE : Modified Duke criteria

- **Major criteria**
  - **Blood culture**
    - **Positive blood cultures (≥2/2) with typical IE microorganisms (viridans streptococci, *S.bovis*, HACEK gp, *S.aureus*, or community acquired enterococci in the absence of primary focus)**
    - **Persistently positive blood cultures defined as 2 culture sets drawn >12 h apart, or 3 or most of 4 culture sets with the first and last separated by ≥ 1 h**
    - **Single positive culture for *C.burnetii* or IgG titre against phase I ≥ 1:800**
  - **Evidence of endocardial involvement**
    - **Positive echocardiogram for IE**

Diagnosis of IE : Modified Duke criteria

- **Minor criteria**
  - **Predisposing conditions**
  - **Fever (T°≥ 38°C)**
  - **Risk factors**
  - **Etc.**
  - **Microbiological evidence:**
    - **Positive blood cultures, but not meeting major criteria; or**
    - **Serological evidence of active infection with plausible microorganism**
  - ***(Echocardiogram consistent with disease but not meeting major criteria)***

Diagnosis of IE : Modified Duke criteria

- **Diagnosis**
  - **Definite**
    - **Pathology or bacteriology of vegetations, major emboli; or**
    - **Intracardiac abscess specimen; or**
    - **Two major criteria; or**
    - **One major and three minor criteria; or**
    - **Five minor criteria**
  - **Possible**
    - **One major and one minor; or**
    - **Three minor criteria**
  - **Rejected**
    - **Firm alternative diagnosis; or**
    - **Resolution after ≤ 4 days antibiotherapy; or**
    - **Etc.**

Diagnosis of IE : Modified Duke criteria

- **Sensitivity**
  - **66 - 100 % in selected cohorts**
- **Limitations**
  - **Negative blood culture**
    - **5 - 20% of cases**
  - **Echocardiography inconclusive**

!

!! RAPID ACCURATE DIAGNOSIS ESSENTIAL !!

- **For early treatment**
  - **Decrease of complication rates - surgery - mortality**

Microbiological diagnosis

- **Conventional culture based**
  - **Blood culture**
  - **Valvular material, embolic tissue, abscess debridement,**
  - **Id & susceptibility**
- **Serological tests**
- **Molecular based techniques**
  - **Identification ; resistance genes**
  - **Specimen**
    - **Blood**
    - **Valvular material**
  - **Isolates from culture**

Microbiological diagnosis

Blood cultures

The most important available laboratory diagnostic test

- **The best method to provide live bacteria**
  - **For identification**
  - **For susceptibility testing**
- **Usually, recovery of etiologic agent**
- **5-20 % of blood culture-negative IE**

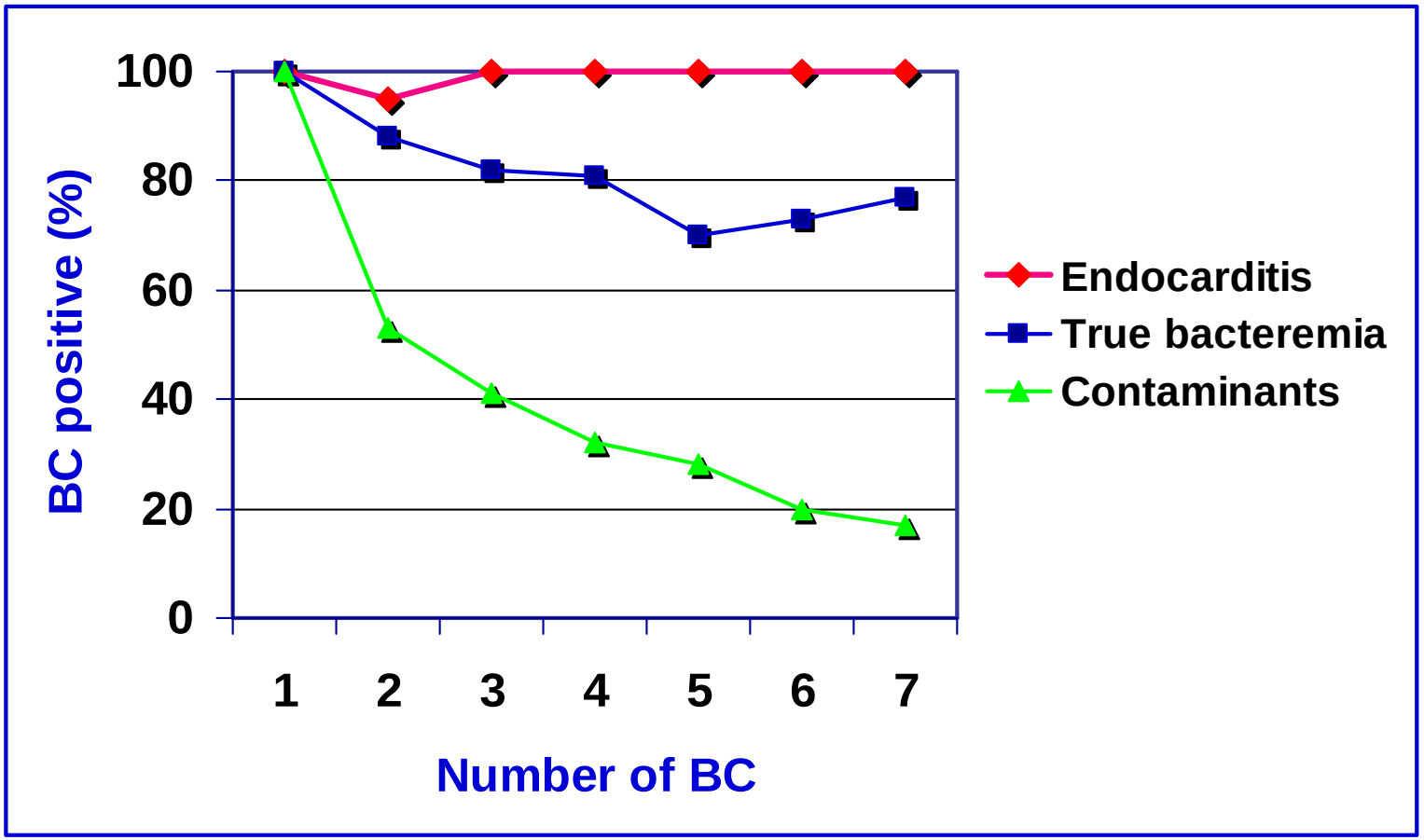
Microbiological diagnosis
Blood cultures

1 - 10 microorganisms/ml until treatment is initiated.

- **Timing**
  - **3 sets**
    - **within first 12-24 h ; > 1 h apart ; different sites**
    - **Or within 2 h if acute IE, before antibiotherapy**
  - **Further blood cultures**
    - **If patient febrile or unwell under treatment**
- **Collection**
  - **Aseptic technique (imperative)**
  - **Arterial blood not superior to venous samples**
  - **Volume : minimum 20 ml/set (adult)**
    - **1 aerobic and 1 anaerobic bottles, or**
    - **2 aerobic bottles**

Microbiological diagnosis
Blood cultures

Patterns of positivity in successive BC evidence of the diagnostic importance of separate cultures (Weinstein et al 1983, Rev InfectDis 5:35-53)



Microbiological diagnosis

Blood cultures

- **Standard**
  - **Automated continuous monitoring systems**
  - **Improved compared to the past**
    - **No one suited for the detection of all blood pathogens**
  - **Improvement**
    - **Media with absorptive resins ...**
  - **Evidence**
    - **Length of incubation**
      - **5 days incubation ; no further prolonged incubation**
- **Specific methods**
  - **Lysis centrifugation techniques**
    - **Prior antibiotherapy**
    - **Fastidious or slow growing microorganisms**

Automated continuous monitoring blood culture system

Microbiological diagnosis
Blood cultures

Prolonged incubation and extensive subculturing do not increase recovery of clinically significant microorganisms from standard automated blood cultures,

EJ Barron, JD Scott, LS Tompkins, CID 2005:41

- **215 patients believed to have had IE**
  - **3 sets of 20ml BC (BACTEC aer+ana)**
  - **Prolonged incubation of culture: 21 days**
    - **Subcultures at days 3 and 10 on 5-6 specific media/bottle**
    - **Acridine orange smear at days 3 and 10**
  - **4 lysis tube, Isolator system**
  - **Only 3 clinically relevant results**
    (2 mycobacteria, 1 *Legionella*)

- **All other patients (14,000 BC)**
  - **2 sets of 20ml BC (BACTEC aer+ana)**
  - **5 days incubation**
    - **Subcultures of positive vials**
  - **24 HACEK group organism recovered**
  - **98% of all positive BC detected by day 4**

Microbiological diagnosis
Blood cultures

Emerging data indicating that extended incubation of blood cultures has little clinical value **MP Weinstein, CID 2005:41**

From

- **Cockerill et al, CID, 2004;38 ; Bhally, Weinstein et al, IDSA , 2004 ; Reller, Duke university, 2005 ; Etc.**

1. **Objective evidence that prolonged incubation of blood cultures for > 5 days is unnecessary for the detection of HACEK bacteria when a modern, automated, continuous-monitoring blood culture system is used**
2. **Extended incubation of BC for patients with suspected IE due to HACEK bacteria or other fastidious organisms has no clinical utility and should not be done**

Microbiological diagnosis
Blood cultures

Emerging data indicating that extended incubation of blood cultures has little clinical value **MP Weinstein, CID 2005:41**

3. Alternatives to extended incubation

- Better diagnostic methods for specific microorganisms
  - Lysis centrifugation tube for dimorphic fungi
  - Special broth media or Lysis centrifugation tube for mycobacteria
  - Molecular amplification for some microorganisms

Microbiological diagnosis
Blood cultures

- **Diagnostic challenge**
  - **Negative blood culture**
    - **5 - 20 % of cases**
    - **Prior antibiotherapy !**
    - **Fastidious or atypical microorganism**
    - **Suboptimal blood collection**
      - **Volume**
      - **Timing**
      - **Temperature**
      - **Transport to the lab and placing in instrument**

Microbiological diagnosis
Blood cultures

Negative blood culture

- **Prior antibiotherapy !**
  - **Recovery of bacteria reduced by 35-40 %**

Washington JAC 1987;20
Hoen et al CID 1995;20
Badour et al Circulation 2005

  - **Recovery of a microorganism is dependent on**
    - **susceptibility of the organism, and**
    - **duration & nature of previous ATB**

Tunkel, Kaye NEJM 1992;20

Microbiological diagnosis
Blood cultures

Prior antibiotherapy

- **Improvement for recovery**
  - **Withholding antibiotics to achieve a positive BC**
    - **No data available**
    - **2 to 4 days if ...**
  - **Special procedures:**
    - **Inactivating antibiotics in culture media**
      - **Dilution to 1:5 or 1:10**
      - **Absorptive resins or activated charcoal particles**
    - **Lysis centrifugation techniques**
    - **PCR amplification 16S ribosomial RNA gene**

Microbiological diagnosis

Blood cultures

- **Negative blood culture**
  - **5 - 20 % of cases**
  - **Prior antibiotherapy !**
  - **Fastidious or atypical microorganism**
    - ***Bartonella* spp; *Tropheryma whippelii*; *Gemella*, *Coxiella*, *Chlamydia*; HACEK group and nutritionally deficient *Streptococcus* spp; *Legionella* spp; mycobacteria**
    - **Fungi, including *C.albicans* and *Aspergillus* spp**

➔ Specific procedures

- **Culture-based**
- **PCR-based**

Microbiological diagnosis

Resected valves or biopsy specimens

- **Conventional cultures**
  - **Infrequently positive (15%)**
  - **Could be helpful and efficient**
    - **Large number of organisms**
  - **False positive**
  - **Use of appropriate medium**
    - **Sometimes guided by positive serologic tests results**
      - ***C. burnetii*, *Brucella* spp, *Legionella* spp, etc**
- **Histologic testing, specific staining**
  - **More reliable indicator of presence of microorganisms**
- **Cell culture**
- **PCR amplification**

Microbiological diagnosis

Antimicrobial Susceptibility Testing

Cornerstone of therapy : bactericidal antibiotics

- **Choice based on AST**
- **Minimum Inhibitory Concentrations (MIC)**
  - **Principal drugs / infecting pathogen**
- **Availability of interpretive criteria**
  - **New guidelines from CLSI for AST of « infrequently isolated or fastidious bacteria »**
    - **CLSI M45-A, May 2006**
    - **Guidance for the most relevant drugs to test and report on specific organisms**

Microbiological diagnosis

AST - CLSI M45-A, May 2006

- **« For infrequently isolated or fastidious bacteria »**

Gram positive

***Abiotrophia* and *Granulicatella* species**
***Bacillus* species (excluding *B.anthracis*)**
***Corynebacterium* species**
Erysipelothrix rhusiopathiae
***Lactobacillus* species**
***Leuconostoc* species**
Listeria monocytogenes
***Pediococcus* species**

Gram negative

Aeromonas hydrophila* complex and *Plesiomonas shigelloides
Campylobacter jejuni/coli
HACEK group
Moraxella catarrhalis
***Pasteurella* species**
***Vibrio* species (excluding *V.cholerae*)**

Microbiological diagnosis

Serologic testing

- **Serologic tests included as diagnostic criteria (Duke)**
  - **For culture negative BC, antibody determination against**
    - ***C.burnetii* *(IF)***
    - ***Bartonella* spp *(IF)***
    - ***Legionella pneumophila* *(IF)***
    - ***Chlamydia* spp *(IF, FC, ELISA)***
    - ***Brucella melitensis* *(agglutination)***
    - ***Mycoplasma pneumoniae* *(ELISA)***

Microbiological diagnosis
Key points

- **Starting IE microbiological diagnostic kit**
  - **3 Blood culture sets (volume !)**
  - **Blood tube for further serological tests**
  - **(Blood tube for further PCR tests)**
- **Standard BC: 5 days incubation**
  - **BC with most microorganisms Positive < 48 h**
  - **98% of Positive BC at 4 days**
- **Team discussion with physician**
- **Saving all blood culture isolates**
  - **Further characterization, comparison or MICs determination**

Conclusions & perspectives

- **Positive blood culture**
  - **Major criterion for diagnosis**
  - **Key to identifying**
    - **Etiologic agent**
    - **Optimal antimicrobial regimen**
- **Culture-negative endorcarditis**
  - **Diagnostic challenge**
  - **Future major criteria??**
    - **Serological tests and PCR-based tests for difficult-to-cultivate organisms**
      - **Strict interpretive criteria**