Laboratory Diagnostics and Biomarkers of Bloodstream Infections: Any News?

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22nd *ECCMID*, London

31 March – 4 April 2012

Laboratory diagnostics and biomarkers of bloodstream infections

Why new tests

Time points

Time 0

- **molecular tests**
- **other tests**

Detection time

- **Maldi-Tof**
- **molecular tests**

Biomarkers

- **steps**

Why new tests

- **Conventional tests (cultures, AST) are slow and/or insensitive (particularly in patients receiving antibiotics)**
- **The prognosis of BSI improves with rapid and appropriate antibiotherapy**

⇒ **need for more rapid tests and/or biomarkers of sepsis**

Different time points for new tests

	before BSI	time 0	detection	identification / AST	
Conventional approaches	**scores**	**clinical signs**	**positive BC (Gram stain)**	**biochemical 24hr**	**48hr**
New tests		**NAT (PCR)**	**Maldi-Tof PNA FISH microarray**	**Maldi-Tof**	
	biomarkers of sepsis (?) →				

NAT : nucleic acid test

Time 0

Direct detection of bacteria in blood or in other body fluids by NAT

- bacterial NA amplification step and human DNA elimination mandatory
- 3 approaches :
  - genus/species specific PCR (MRSA *N.meningitidis* *S.pneumoniae*)
  - multiplex PCR (20-30 most predominant organisms)
  - eubacterial PCR followed by sequencing

Commercial molecular tests for BSI detection at time 0

Product	Volume (ml) of blood	DNA enrichment	DNA detection	Bacterial species	Fungi
Septifast Roche	**1.5**	**-**	**fluorescent probes**	**19**	***Candida* spp (5)** ***Aspergillus fumigatus***
LOOXTER/VYOO SIRS-Lab	**5**	**via affinity chromatography**	**gels**	**40**	***Candida*** ***Aspergillus*** **panfungal**
Sepsi Test Molzym	**5**	**selected lysis**	**gels, sequencing**	**>300**	***Candida*** ***Cryptococcus***
PLEX-ID BAL Abbott-Ibis	**1.5**	**-**	**Mass spectro**	**>300**	***Candida* spp (9)**

BSI detection at time 0 – Light Cycler Septifast Test

To date, the only multiplex real-time commercial PCR assay for the diagnosis of sepsis

Detects 25 bacterial and fungal pathogens (target : ITS regions)

Mechanical DNA extraction

3 parallel multiplex real-time PCR reactions

Detection limit : 3-30 CFUs/ml

TAT 6h

> 20 studies in neutropenic, pediatric, ICU, ER patients

BSI detection at time 0 – Light Cycler Septifast Test

4 studies, 2 with more than 100 episodes of febrile neutropenia (FN)

	FN episodes without antibiotics			FN episodes with antibiotics		
	n=	+BC	+SF	n=	+BC	+SF
Von Lilienfeld M	119	36%	24%	?	3%	15%
Lamoth F	141	31%	33%	52	8%	37%

1) von Lilienfeld M et al, *J Clin Microbiol* 2009; 47: 2405
2) Lamoth F et al, *J Clin Microbiol* 2010; 48: 3510.

BSI detection at time 0 – Light Cycler Septifast Test

Pros and Cons

⊕

Proof of concept

Additional value to blood cultures

- in patients on antibiotics
- in patients with suspected invasive fungal infection

moderately rapid

⊖

Low sensitivity

- low volume of blood
- low DNAemia

False negative

- not included in the spectrum

False positive?

- no gold standard
- contamination

work-intensive

high cost

lack of automation

cannot replace blood cultures

BSI detection at time 0 – Plex-ID-Bal®

PCR coupled to electrospray ionization mass spectrometry **(PCR/ ESI-MS)**

(broad range primers and strains-specific primers, antibiotic resistance genes)

Mass/charge (m/z) ratio of the amplicons measured by mass spectrometry

No prior knowledge of an organism necessary

> 96% concordance with classic methods in positive BC broth

Can identify mixture of organisms

TAT 5-6 h, costly, need batch testing (current version).

Kaleta EJ et al, *Journal of Clinical Microbiology* 2011; 49: 345.

Detection time – Direct and rapid identification from positive BC

Commercially available assays

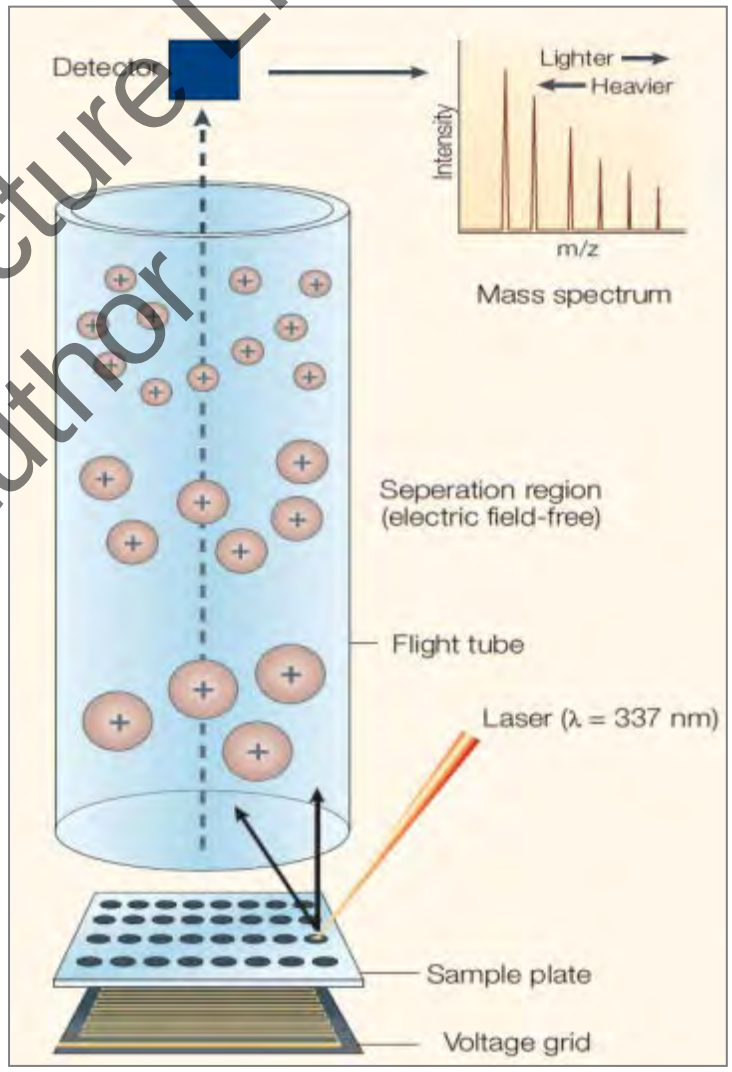
Assays	Format	Pathogens detected	TAT
PNA-FISH (advan Dx)	**fluorescent based hybridization**	**bacteria yeast**	**2-3 h**
Prove-it sepsis (Mobidiag)	**multiplex PCR + hybridization on a microarray**	**50 bacteria**	**3 h**
GenXpert	**single species PCR**	**MRSA/SA**	**1.5 h**

Adapted from **Mancini N et al, *Clin Microbiol Rev* 2010; 23: 236.**

Detection time – Direct and rapid identification from positive BC

Maldi-Tof

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)



Digital genotyping using molecular affinity and mass spectrometry, Sobin Kim, Hameer D. Ruparel, T. Conrad Gilliam and Jingyue Ju. Nature Reviews Genetics – Vol 4, December 2003

Direct testing of positive blood cultures by Maldi-Tof

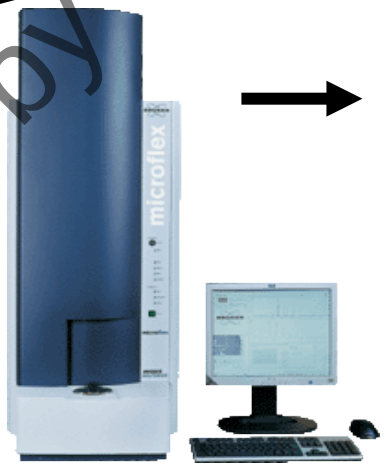
Sampling

Incubation of blood culture bottles

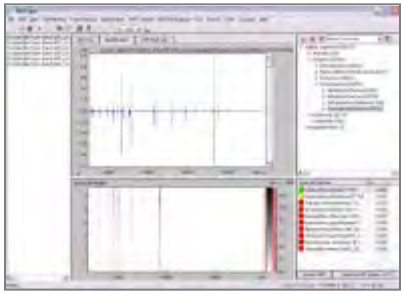
WHEN POSITIVE

Preparation of a bacterial pellet

Deposition of bacterial pellet on MALDI microplate



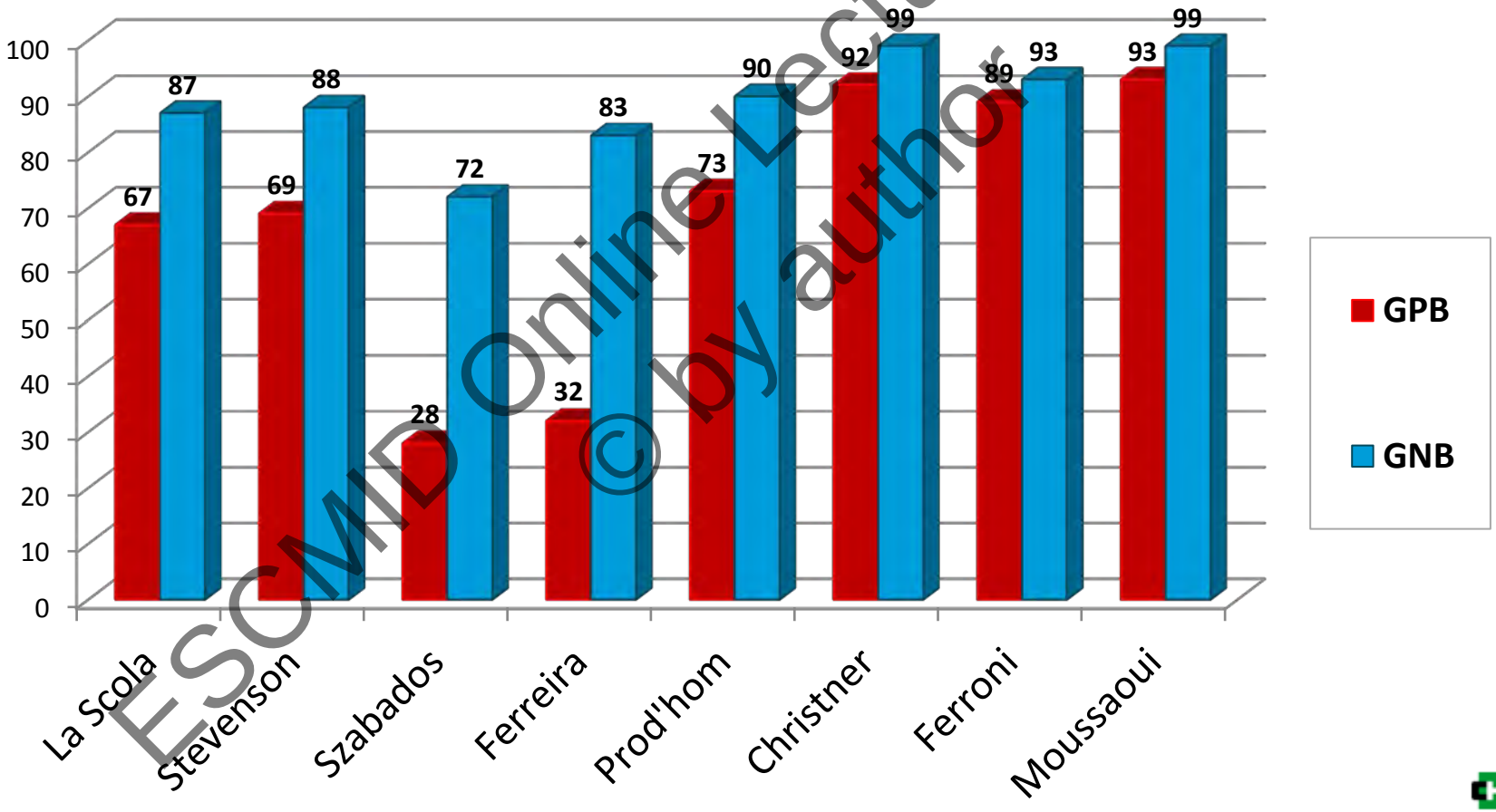
Acquisition of the proteic profile



Comparison with a database

Comparative performance for Gram positive and Gram negative organisms

(8 published studies of mono-microbial bacteremia)



Maldi-Tof MS in your clinical microbiology laboratory – Pros and Cons

⊕	⊖
Rapidity	- cost of the instrument
Ease to use	- misidentifications (rare) *S.mitis – S.pneumoniae* *Shigella – E.coli*
Cost per test	
Accuracy of identification	- not applicable to native specimens
Reproducibility	- cross-contamination
Access to database	- mixed culture
Prospect for typing	- lack in the commercial database (filamentous fungi)
	- limited application to antibiotic susceptibility testing

Detection time – Direct and rapid AST from positive BC

- **Commercial "POCT-PCR"**
  (GenXpert MRSA, VRE)
- **DNA microarray**
  (check-MDR CT101)
  AmpC/ KPC/ NDM-1 gene assay

Biomarkers

Time points and uses

- **Screening of patients at increased risk**
- **Diagnosis**
- **Risk stratification**
- **Monitoring response to therapy**

Definition

"a quantifiable measurement of biological homeostasis defining what is normal and what is abnormal"

Adapted from **Marshall JC and Reinhart K, for the International Sepsis Forum, *Crit Care Med* 2009; 37: 2290.**

Sepsis biomarkers : a review

Key messages:

- **More than 170 different biomarkers assessed for potential use in sepsis, more for prognosis than for diagnosis**
- **None has sufficient specificity or sensitivity to be routinely employed in clinical practice**
- **Combination of several biomarkers may be more effective than single biomarkers, but this requires further evaluation.**

Adapted from **Pierrakos C, Vincent JL, *Crit Care Med* 2010; 14: 815.**

Sepsis biomarkers : a review

Categories of biomarkers:

Cytokines/ chemokines	**18**
Cell markers	**14**
Receptors	**17**
Coagulation	**8**
Endothelium activation	**15**
Vasodilatation	**15**
Organ dysfunction	**17**
Acute phase proteins	**9**
Others	**…**

Adapted from **Pierrakos C, Vincent JL, *Crit Care Med* 2010; 14: 815.**

Sepsis biomarkers : a review

Biomarkers for use in the diagnostic of sepsis **(34)**

6 with sensitivity and specificity > 90%

- **aPTT** **(activated partial thromboplastin time) high NPV**
- **CD 11b** **in neonates**
- **CD 64**
- **IL-12** **in pediatric patients**
- **IP-10** **(interferon-induced protein) in neonates**
- **PLA 2-II** **(phospholipase A2)**

Adapted from **Pierrakos C, Vincent JL, *Crit Care Med* 2010; 14: 815.**

Sepsis biomarkers : a review

Biomarkers specially evaluated for the early diagnosis of sepsis

16 factors

5 with sensitivity and specificity > 90%

- **IL-12** **in newborns, not in adults**
- **IP-10** **in neonates** (sepsis and NEC), **not in adults**
- **PLA 2-II** **in critically ill bacteremic adults**
- **CD 64** **in adults**
- **Neutrophil CD 11b** **in children**

Adapted from **Pierrakos C, Vincent JL, *Crit Care Med* 2010; 14: 815.**

Sepsis biomarkers : a review

Biomarkers with high negative predictive value to rule out sepsis

	NPV	
PCT	**99%**	**at a cut-off value of 0.2 μg/ml**
aPTT	**96%**	
Fibrin degradation products	**100%**	**for Gram-negative sepsis**

Sepsis biomarkers : a review

Promising biomarker for the diagnosis of sepsis

- **Angiopoeitin–1/2 system (Ang-) -endothelial activation**
  - **consistent association with sepsis**
  - **no cut-off for its clinical use**

Sepsis biomarkers : a review

Future "biomarkers" for the diagnosis of sepsis

Microarray approaches

Genome-level signatures able to distinguish:

- **SIRS from sepsis: ribonucleogram for the early prediction of VAP (Cobb[a])**
- ***S.aureus* from *E.coli* infection (Ramilo[b])**

a) Cobb JP et al, *Ann Surg* 2009; 250: 531
b) Ramilo O et al, *Blood* 2007; 109: 2066.

Review: **Sepsis and Septic Schock – the Potential of Gene Array**
Wong HR, *Critical Care* 2012; 16: 204.

Sepsis biomarkers : a review

Current use of biomarkers for the diagnosis of sepsis

No single new marker with sensitivity and specificity high enough to warrant clinical use

Use clinical judgment, scores, and CRP/PCT with moderation

Watch up for the next ECCMID !