New Approaches in Diagnosing Sepsis

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Outline

- Introduction
- Sepsis bundles
- Diagnosig sepsis
  - Molecular methods
- Experience from Kuwait

Introduction

Definition of sepsis

Sepsis **is present, when an infectious focus has formed in the body from which the germs are disseminated and spread, so that a systemic response can be observed in peripheral organs!“**

SEPSIS

=

Infection

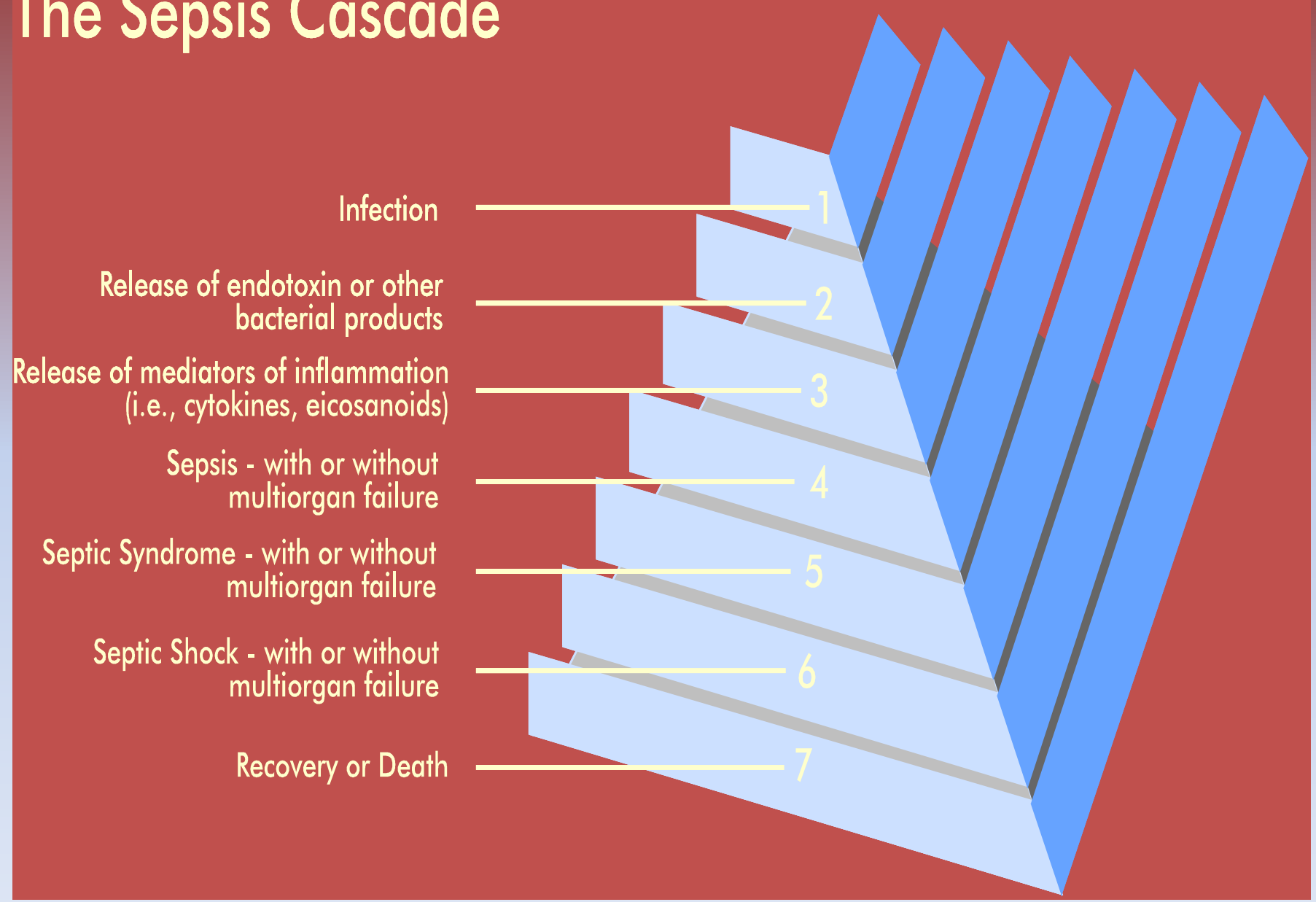
+

Systemic inflammation

Every 3-4 seconds someone dies of sepsis

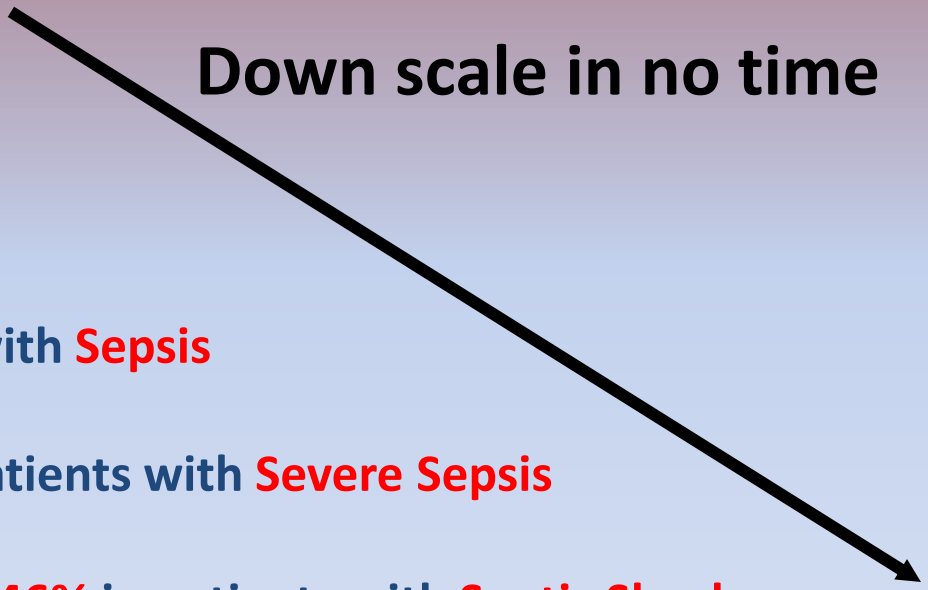
Stop it
Spot it

The Sepsis Cascade



Mortality rate increases with increasing severity

Mortality rate is:



- 7% in patients with SIRS
- 16% in patients with Sepsis
- 20% in patients with Severe Sepsis
- 46% in patients with Septic Shock

Rangel-Frausto et al. (JAMA 1995)

The World Sepsis Day

13th September


Diagnosing
Sepsis

We ought to spend more time to search for an accurate diagnosis rather than search for the Magic Bullet for the treatment of Sepsis"

Roger Bone, 1996

The Golden Hour

And

The Silver Day

- **Resuscitation bundle**
- **6 hours**

Golden hours

- **Management bundle**
- **24 hours**

Silver day

How can we improve our laboratory service in the diagnosis of Sepsis??

DNA-based techniques

Rapid identification (within 2 hrs) after a positive blood culture signal

Evaluation of the comperative performance of Verigine Blood Culture Nucliec acid System to Conventional Techniques in a Tertiary-care Hospital in Kuwait

*Mokaddas EM, Behbehani A, Abdullah A, Shatti S

- The Verigene Gram-positive and Gram negative Blood Culture (BC-GP, BC-GN)) system (Nanosphere, USA) is a qualitative multiplexed automated nucleic acid in vitro diagnostic test for the direct identification of Gram-positive and Gram negative bacteria and their genetic resistance markers.

Verigene BC-GP and BC-GN identifiable targets

Gram-Positive Blood Culture (BC – GP) Tests :

Genus	**Staphylococcus Spp.** **Streptococcus Spp.** **Micrococcus Spp.** **Listeria Spp.**	**Species**	**S. aureus** **S. epidermidis** **S. lugdumesis**
			S. pneumoniae S. anginosus. Group S. agalaticae
Resistance	Mec A. Van A. Van B.		S. pyogenes Enterococcus faecalis Enterococcus faecium

NB. Of the staphylococci only *S.aures, S. epidermidis* and *S. lugdumesis* can be identified as the other staphylococci are not present in the data base.

Targets	Organism/Gene	
Bacterial Targets	*Acinetobacter* spp.	
	Citrobacter spp.	
	Enterobacter spp.	
	Proteus spp.	
	E. coli	
	Klebsiella pneumoniae	
	Klebsiella oxytoca	
	Pseudomonas aerogenes	
	Serratia marcescens	
Resistance Marker	CTX-M	VIM
	KPC	IMP
	NDM	OXA (48/23/40/58)

N.B. *Stenotrophomonas maltophilia* cannot be identified as it is not present in the data base

Objectives

- **To evaluate the performance of Verigene (BC-GP and BC-GN) nucleic acid test for the direct identification of Gram-positive and Gram-negative bacteria from positive blood culture bottles in comparison with Gene–Xpert system (Cephide, USA) for Gram-positive bacteria and with the conventional culture technique for both Gram-positive and Gram-negative bacteria.**
- **To evaluate the performance of Verigene (BC-GP) for the detection of resistant markers directly from positive blood culture bottles in comparison with conventional culture technique.**
- **To evaluate the performance of Verigene (BC-GN) for the detection of resistant markers directly from positive blood culture bottles in comparison with conventional culture technique.**

Materials and Methods

- All the demographic data including the age, sex, patient location, underlying clinical condition, clinical and laboratory data suggesting sepsis, initial empirical therapy, adjusted therapy and outcome of the patients were collected.
- **For Gram-positive bacteria:**
  - All blood culture bottles (Bactec, Bekton Dickinson, USA) showing Gram-positive cocci by Gram stain were processed in:
    - Verigene for BC-GP according to the manufacturer's instructions
    - GeneXpert (Cepheid, USA) for BC-GP (only for Gram-positive cocci in clusters)
  - All the positive blood culture bottles were simultaneously cultured by conventional methods for both ID as well as susceptibility using Vitek II, and Vitek MS (Biomerioux, France)

Materials and Methods

- **For Gram-negative bacteria:**
  - All blood culture bottles showing Gram-negative bacilli by Gram stain were processed in:
    - Verigene for BC-GN according to the manufacturer's instructions
  - All the positive blood culture bottles were simultaneously cultured by conventional methods for both ID as well as susceptibility using Vitek II, and Vitek MS
- A total of 11 QC strains of different streptococci were included in the evaluation

Results

A. Gram-positive

A total of 63 patients with positive blood culture for Gram-positive cocci were included in the evaluation

Table 1: Comparison between results of Verigine and conventional culture for Gram-positive bacteria

Gram-positive	Virigine	Conventional culture
Staphylococcus aureus	16	16
S.epidermidis	19	17
S.homonis	0	1
S.hemolyticus	0	3
Other Staphylococci	9	6
Enterococcus fecalis	9	9
Enterococcus fecium	4	4
Streptococcus pneumoniae	2	2
Streptococcus mitis	1	2
Streptococcus spp.	2	1
Micrococcus spp.	1	0

Table 2: Comparison between results of Verigine and conventional culture for 11 QC strains

Gram-positive cocci (QC strains)	Verigine	Conventional	% Concordance
Streptococcus pneumoniae	3	3	100
Streptococcus agalactiae	4	4	100
Streptococcus pyogenes	3	3	100
Enterococcus fecium	1	1	100

Table 3: Comparison between Verigine, Cephid Gene Xpert and conventional culture for *Staphylococcus* spp.

Staphylococcus spp.	Verigine	Gene Xpert	Conventional culture	% Concordance
Methicillin sensitive *Staphylococcus aureus*	8	8	8	100
S.epidermidis	12	12	12	100
S.homonis	1	1	1	100
S.hemolyticus	2	2	2	100

Table 4: Comparison between Verigine and conventional culture for detection of reistance markers for *Staphylococcus* spp.

Conventional Culture	Verigine	
	Mec A negative	Mec A positive
Methicillin sensitive *staphylococcus aureus*	TN 11	FP 2
MRSA	FN 2	TP 1
Methicillin-resistant coagulase-negative Staphylococci	0	TP 15

Table 5: Comparison between Verigine and conventional culture for detection of reistance markers for *Enterococcus* spp.

	Verigine	
Conventional Culture	**VAN A and B negative**	**VAN A and B positive**
Vancomycin sensitive *Enterococcus fecalis*	**TN 9**	**0**
Vancomycin-sensitive *Enterococcus fecium*	**2**	**0**
Vancomycin-resistant *Enterococcus fecium*	**FN 2**	**0**

A total of 63 patients with positive blood culture for Gram-negative bacilli were included in the evaluation

Table 6: Comparison between results of Verigine and conventional culture for Gram-negative bacteria

Gram negative	Verigine	Conventional culture	% Concordance
E.coli	24	24	100
Acinitobacter spp.	15	15	100
Klebsiella pneumoniae	8	8	100
Pseudomonas aeruginosa	7	7	100
Pseudomonas oryzihabitans	1	1	100
Enterobacter spp.	2	2	100
Proteus spp.	1	1	100
Serratia marcescens	1	1	100

Table 8: Impact of rapid identification of Gram-positive bacteria on the modification of the empirical antibiotic

Antimicrobial stewardship

Gram positive bacteria			same otic	Stop antibiotic
Staphylococcus spp.	11		16	14
Enterococcus spp.	0	0	13	0
Streptococcus pneumoniae	0	0	2	0
Streptococcus mitis	0	0	0	1

Table 9: Impact of rapid adentification of Gram-negative bacteria on the modification of the empirical anti[illegible]y

Antimicrobial stewardship

Gram- negative bacte			Continue same antibiotic
Enterobacteriacae			21
Pseudomonas aeruginosa	0	3	4
Acinitobacter spp.	0	6	9

Conclusion

- Verigene BC can h… **…and rapid identification of Gram-positi… …and their resistance marker… …ttles.**
- The **tr**… …ection of resist… …g conventional culture…
- **Modifica**… …llowing the rapid identification … …th their resistance markers directly from positive blood cultures
- Rapid molecular methods used to identify both Gram-positive and Gram-negative bacteria directly from positive blood culture bottles in septic patients greatly helps the implementation of antimicrobial srtewardship programs in the process to encourage rational use of antimicrobial agents with subsequent reduction in antibiotic resistance as well as cost.

Will be introduced into Kuwait by end of the year

Acknowledgement

Conclusion

Diagnosing Sepsis

Tomorrow is too late!!!

Sepsis is an Emergency

Sepsis in a potentially fatal condition caused by the body's immune system

This is more than lung cancer And more than breast cancer and colon cancer combined

Sepsis claims the lives of 37,000 people in the UK each year

Sepsis is not a condition that is spoken about a lot

When find out the full extent of the severity of it, it is fearing

Thousands dying of sepsis because of poor NHS care

Its Time to act

The delays are causing almost 13,000 deaths of sepsis needlessly a year (UK)

Unfair while we are in the 21st century to carry [illegible] tests that

Tomorrow is too late

Even for 24 hrs

Thank you