Clinical Mg of dermohypodermitis and necrotizing fasciitis

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Necrotizing soft tissue infections (NSTIs)

Rare disease: 4/100000 people
High mortality rate: 20-50%
NSTIs Rx management

NONPURULENT
Necrotizing Infection /Cellulitis /Erysipelas

- Severe
- Moderate
- Mild

EMERGENT SURGICAL INSPECTION / DEBRIDEMENT
- Rule out necrotizing process

EMPIRIC Rx
- Vancomycin PLUS Piperacillin/Tazobactam

C & S

DEFINED Rx (Necrotizing Infections)
Monomicrobial Streptococcus pyogenes
- Penicillin PLUS Clindamycin
- Clostridial sp.
- Penicillin PLUS Clindamycin
- Vibrio vulnificus
- Doxycycline PLUS Ceftazidime
- Aeromonas hydrophila
- Doxycycline PLUS Ciprofloxacin

Polymicrobial
- Vancomycin PLUS Piperacillin/Tazobactam

INTRA VENOUS Rx
- Penicillin or
- Ceftriaxone or
- Cefazolin or
- Clindamycin

ORAL Rx
- Penicillin VK or
- Cephalexin or
- Dicloxacillin or
- Clindamycin

MANAGEMENT OF SSTIs

PURULENT
Furuncle / Carbuncle / Abscess

- Severe
- Moderate
- Mild

EMPIRIC Rx
- Vancomycin or
- Daptomycin or
- Linezolid or
- Televancin or
- Ceftaroline

EMPIRIC Rx
- TMP/SMX or
- Doxycycline

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DEFINED Rx
MRSA
- See Empiric
- MSSA
- Nafcillin or
- Cefazolin or
- Clindamycin

DEFINED Rx
- MRSA
- TMP/SMX
- MSSA
- Dicloxacillin or
- Cephalexin

\[\text{\textsuperscript{1}}\text{Since daptomycin and televancin are not approved for use in children, vancomycin is recommended; clindamycin may be used if clindamycin resistance is <10-15% at the institution.}\]
# Microbial classification of NSTIs

<table>
<thead>
<tr>
<th>Classification</th>
<th>Etiology</th>
<th>Pathogens</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I (70-80%)</td>
<td>Polymicrobial Gut microbiota</td>
<td>Aerobic/anaerobic Enterobacteria/Enterococcus sp.</td>
<td>Depending on comorbidities (Mortality from 5% to 50%)</td>
</tr>
</tbody>
</table>
| Type II (20-30%)   | Monomicrobial Skin/ENT microbiota | S. pyogenes  
S. aureus  
other Streptococcus beta-hemolytic | Mortality > 30% - More if myositis is associated                           |
| Type III (infrequent) | Gram negative Hydric bacteria | Vibrio spp.  
Haemophilus sp. | Mortality : 30 to 40%                                                   |
| Type IV (very infrequent) | Yeast/Mucorales (frequently post-traumatic) | Candida spp. in Immunocompromised  
Zygomycetes in Immunocompetent | Mortality > 50%  
More in immunocompromised                                                   |

*Morgan MS et al, J Hosp Infect. 2010*
Aim of the study

• Establish a map of microbial diversity of NSTIs by different approaches including NGS 16S and Shotgun Mg

• Evaluate different technical approaches for diagnostic

• Evaluate interest of these approaches in clinical routine
Patients

- NSTIs reference center in Henri-Mondor Hospital
- 34 patients enrolled in the study: clinical, radiological and microbiological data
- Empiric antibiotherapy
  - Piperacilline-Tazobactam (4 g x 3 -4/ day) + Clindamycine (600 mg x 3-4/day adapted to weight)
- Surgery for all patients INCLUDING biopsy
  - necrotic skin area (N=34)
  - healthy skin area (N=10)

### Criteria

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>62 [24-93]</td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>0,44</td>
</tr>
</tbody>
</table>

### Pathology

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH</td>
<td>5</td>
</tr>
<tr>
<td>NF</td>
<td>28</td>
</tr>
<tr>
<td>Myositis</td>
<td>1</td>
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</table>

### Comorbidity

<table>
<thead>
<tr>
<th>Comorbidity</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>13</td>
</tr>
<tr>
<td>Immunocompromised</td>
<td>10</td>
</tr>
<tr>
<td>Obesity</td>
<td>9</td>
</tr>
<tr>
<td>Toxic (alcohol, drugs, NSAIDs)</td>
<td>6</td>
</tr>
</tbody>
</table>

### Clinical context

<table>
<thead>
<tr>
<th>Clinical context</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open wound (traumatic)</td>
<td>8</td>
</tr>
<tr>
<td>Chronic wound</td>
<td>11</td>
</tr>
<tr>
<td>Post chirurgical</td>
<td>1</td>
</tr>
<tr>
<td>Other (Bite, intertrigo...)</td>
<td>4</td>
</tr>
<tr>
<td>Unknown</td>
<td>10</td>
</tr>
</tbody>
</table>

### Antibiotherapy

- Pre-admission: 23
- Post admission before surgery: 34
- Respecting guidelines: 33

### Management and follow-up

- Number of surgery (median): 1 [1-4]
- Intensive Care admission/shock: 17
- Death: 2
Methods

**Culture**
- TSA
- Drigalsky
- CAN
- Columbia Aerobic and anaerobic
- Schaedler broth
- Chocolate (5%CO₂)

**DNA/RNA extraction**
- DNA/RNA Special protocol for metagenomic

**MALDI-TOF MS**
- Fungi and Bacteria Identification

**Targeted Metagenomic (TM)**
- 16S/ITS Lib prep
- MiSeq Sequencing
- Fungi and Bacteria Identification analysis

**Shotgun Metagenomic (SM)**
- DNA Lib prep (Nextera XT) + RNA Lib prep (Total RNA)
- NextSeq Sequencing
- MetaMIC® All pathogens Identification and quantification analysis

*Sitterle et al., Front in Microbiol 2017

Sample biopsy containing Bacteria, fungi, and/or viruses
Focus on Shotgun method « Mondor »

Automatized and standardized Pathogen genome Extraction (12 samples per run)

Library preparation DNA+RNA

Sequencing (Illumina NextSeq)

Server calculation (96Co/4To RAM)

MetaMIC®

Filtering

Classify and quantify with DB

Report and interpret
Qualitative Results (I)

More polymicrobial samples comparing to 16S

Less negative samples

TM : Targeted Metagenomic (16S+ITS)
SM : Shotgun Metagenomic
Qualitative Results (II)

- Pathogens isolation number in whole cohort:
  - Negative
  - Anaerobic
  - Enterobacteria
  - S. aureus
  - Other Staphylococcus
  - S. pyogenes
  - Other Streptococcus
  - Corynebacterium sp.
  - Fungi

- Number of samples:
  - Better with SM
  - Better with TM
  - Better with Culture
  - Equal Culture/SM
  - Equal Culture/TM/SM
  - Negative

* (p<0.01)
Quantitative Results (I)

$ r = 0.62 $  
$p < 0.001$
Quantitative Results (II)

- Healthy Area
- Necrotic Area

SM ratio bacteria/human

- P < 0.01

Graph showing the comparison between Healthy Area and Necrotic Area with a significant difference indicated by P < 0.01.
Conclusion

• Shotgun Metagenomic is
  – always better than 16S in NSTIs diagnostic
  – better than culture for hard to culture bacteria (anaerobic, environmental gram negative)
  – Usable for bacteria quantification
  – Can distinguish different medical situation (healthy/necrotic situation)

• Shotgun is not better than culture for easy to culture bacteria

• Shotgun is usable in medical diagnostic
Many thanks

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