If it’s on the pitch, is it interfering with play?

Craig Williams
There are lots of bugs in ulcers!

Time of report 24/10/2017 1613

PUDDLEDUCK, JEMIMA (D410869)
DOB 16/11/2007 (9Y)  Sex F
NHS No.:

Hospital ID WDGH
Location
Att phys 1
Att phys 2

T81389 Collect D/T: 24/10/2017 0900  Receive D/T: 24/10/2017 1530
Order account #: D410869-TEST  Order location: UNKO

Order physician:
Wound Routine culture
Specimen Description
Special Requests
Culture
Wound swab
testing
Staphylococcus aureus heavy growth.
Pseudomonas aeruginosa moderate growth.
Coliform moderate growth.
Enterococcus species moderate growth.

Report Status
Final 24/10/2017

Susceptibility
Organism
Staphylococcus aureus heavy growth.
Method
Stokes
Erythro/Clarithromycin
Susceptible
Flucloxacin
Susceptible

*** END OF REPORT ***
If it’s on the pitch, is it interfering with play?

If a player is not interfering with play then he shouldn't be on the pitch.

— Brian Clough —
The difference between microbiology and cardiology

Doubt is not a pleasant condition, but certainty is absurd

Voltaire
Diabetic foot ulcers are a problem

More than 60,000 people with diabetes in England are thought to have foot ulcers at any given time.

In 2014-15 the annual cost of diabetic foot disease to the NHS in England was estimated at £1 billion, in addition to the personal/social costs of reduced mobility and sickness absence.

Only around half of people with diabetes who have had a diabetic foot ulcer survive for 5 years.

Treatment for diabetic foot disease may involve amputation. Around 7,000 people each year.

National Diabetes Foot Care Audit - 2014-2016
### Table 8: Factors associated with being alive and ulcer-free at 12 and 24 weeks, England and Wales, 2014-2016

<table>
<thead>
<tr>
<th>Factors associated with healing</th>
<th>Outcome at ___ weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Patient is female</td>
<td>▲</td>
</tr>
<tr>
<td>Patient is from a less deprived area of the country</td>
<td>▲</td>
</tr>
<tr>
<td>Patient has Black or Asian ethnicity</td>
<td>▲</td>
</tr>
<tr>
<td>Patient has Type 1 diabetes</td>
<td>▲</td>
</tr>
<tr>
<td>Patient has had diabetes for less than 5 years</td>
<td>▲</td>
</tr>
<tr>
<td>Patient has had diabetes at least 5 and less than 10 years</td>
<td>▲</td>
</tr>
<tr>
<td>Patient self-referred to the specialist foot care service</td>
<td>▲</td>
</tr>
<tr>
<td>Patient has mixed or ‘other’ ethnicity</td>
<td>▼</td>
</tr>
<tr>
<td>Patient currently smokes</td>
<td>▼</td>
</tr>
<tr>
<td>Patient presented with Charcot foot disease</td>
<td>▼</td>
</tr>
<tr>
<td>…with Site/Ischaemia/Neuropathy/Area/Depth</td>
<td>▼</td>
</tr>
<tr>
<td>…with Bacterial infection</td>
<td>▼</td>
</tr>
<tr>
<td>Patient waited more than 2 months for expert assessment</td>
<td>▼</td>
</tr>
<tr>
<td>Patient has not had all 8 NICE recommended processes</td>
<td>▼</td>
</tr>
</tbody>
</table>

**Key:** Strength of models (c-statistic) = poor. See Glossary (Statistical terms) in the main report for explanation of terms.

▲ = Associated with better healing; ▼ = associated with worse healing; ◄► = no association found. Tested at the 0.05 level.
Table 10: Ulcer factors associated with ulcer healing at 12 and 24 weeks, England and Wales, 2014-2016

<table>
<thead>
<tr>
<th>Ulcer characteristic</th>
<th>12 weeks</th>
<th>24 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strength of model (c-statistic)¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>0.697</td>
<td>Poor</td>
</tr>
<tr>
<td>Odds ratios¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SINBAD element: Site (on hindfoot)</td>
<td>▼ 0.810</td>
<td>▼ 0.702</td>
</tr>
<tr>
<td>SINBAD element: Ischaemia</td>
<td>▼ 0.494</td>
<td>▼ 0.482</td>
</tr>
<tr>
<td>SINBAD element: Neuropathy</td>
<td>▼ 0.637</td>
<td>▼ 0.756</td>
</tr>
<tr>
<td>SINBAD element: Bacterial infection</td>
<td>▼ 0.792</td>
<td>▼ 0.594</td>
</tr>
<tr>
<td>SINBAD element: Area (≥1cm²)</td>
<td>▼ 0.504</td>
<td>▼ 0.594</td>
</tr>
<tr>
<td>SINBAD element: Depth (to tendon or bone)</td>
<td>▼ 0.670</td>
<td>▼ 0.684</td>
</tr>
<tr>
<td>Charcot foot disease = present²</td>
<td>▼ 0.722</td>
<td>▼ 0.620</td>
</tr>
<tr>
<td>Charcot foot disease = possible²</td>
<td>▲ 1</td>
<td>▲ 1</td>
</tr>
<tr>
<td>Charcot foot disease = unknown²</td>
<td>▲ 1</td>
<td>▲ 1</td>
</tr>
<tr>
<td>Time to expert assessment = self-referred³</td>
<td>▲ 1</td>
<td>▲ 1</td>
</tr>
<tr>
<td>Time to expert assessment = &gt;2 months³</td>
<td>▼ 0</td>
<td></td>
</tr>
</tbody>
</table>

Notes: 1. See Glossary (Statistical terms) in the main report for explanation of terms. 2. Vs. Charcot foot disease = not present. 3. Vs. Time to expert assessment = ≤2 days.

Key: Strength of models (c-statistic) = poor. See Glossary (Statistical terms) in the main report for explanation of terms. ▲ = Associated with better healing; ▼ = associated with worse healing; ◄► = no association found. Tested at the 0.05 level.
There are lots of bugs everywhere!
Especially on the skin

Are diabetics different?

The diabetic microbiota

phylum Actinobacteria, \textit{(Corynebacterium)}, is more prevalent in diabetic foot skin

No follow up so no extrapolation to possible DFU prognostic microbial signatures was attainable with this study.

\textit{Redel J Inf Dis} 2013 Apr;207(7):1105-14
Is there a bad microbiome?

Each ulcer is an experiment in bacteriology.
Don’t forget the fungi

Image of a scientific graph showing fungal diversity and distribution. The text in the image reads: "A) Proportion of fungal taxa at baseline visit. B) Shannon Index for forefoot, midfoot, and hindfoot. C) Mean proportion of fungal taxa across locations. D) Mean weighted UniFrac between and within subjects."
So far

- Complex mixture of bacteria and fungi
- No smoking gun so far
How do organisms grow in ulcers

Biofilms are present in most, if not all, chronic non-healing wounds. 78% of chronic wounds contain a biofilm. Micro-organisms in biofilms are not only located at the wound surface but may also be present in deeper tissues.

Malone J Wound Care 2017;26:20-5
Schaber Infect Immun 2007;75:3715-21
How is the biofilm growing?

Streptococci  Actinomyces naeslundii

Functionally Equivalent Pathogroup: consortia of genotypically distinct bacteria that symbiotically produce a pathogenic community.

J Clin Micro 2008, 8:2717-22
What does competition mean?

• Mixtures in biofilms behave differently to planktonic mixtures
  • Staph aureus and Pseudomonas

• Biofilms overall develop to reduce immunogenicity

• Complex inter-kingdom and inter-species interactions occur in biofilm
Bacterial interaction: Staph and Pseudomonas

Protein A from *S. aureus* protects *P. aeruginosa* from neutrophil phagocytosis

Armbruster et al. mBio 2016; doi:10.1128/mBio.00538-16
Ps aeruginosa isolates from coinfected CF patients are less competitive with S. aureus.
Inter-kingdom interactions: Candida and Staph

Kean. Front Microbiol. 2017 Feb 23;8:258
Inter-kingdom interactions: Candida and Staph
Bacterial interactions in biofilms: Mouse model

Mixed infections delay healing

Does the "immunogenicity" of the mixed biofilm change over time?

The middle period (12 weeks) seems to be important.

So far

• The bugs grow as biofilms, probably in discrete clumps of “competing” bacteria in “unhappy” biofilms

• Bacterial species can
  • interact to modify host response
  • Interact with each other to allow other species to thrive
  • Interact with fungi to increase pathogenicity
This is all very complicated
The hydrogel-cellulose matrix (3D) model

C the cellulose matrix containing the microorganisms

B 50% serum gel

Allows the form of a 3D biofilm structure.
2D v 3D model

Individual organisms grown on Thermanox coverslips (2D)

Individual organisms grown in Cellulose matrix (3D)

Townsend Biofouling 2016 Nov;32(10):1259-1270
2D v 3D model with antiseptics

Townsend Biofouling 2016 Nov;32(10):1259-1270
Viability in 2D and 3D systems
3D model with antimicrobials

Flucloxacillin
Ciprofloxacin
Fluconazole
<table>
<thead>
<tr>
<th></th>
<th>FLX</th>
<th>CIPX</th>
<th>FLC</th>
<th>FLX FLC</th>
<th>FLC CIPX</th>
<th>FLX CIPX</th>
<th>FLX CIPX FLC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SA</strong></td>
<td>1.33</td>
<td>3.61</td>
<td>1.26</td>
<td>0.39</td>
<td>1.19</td>
<td>1.72</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>CA</strong></td>
<td>1.73</td>
<td>0.87</td>
<td>0.09</td>
<td>1.13</td>
<td>2.11</td>
<td>3.58</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1.53</td>
<td>1.14</td>
<td>0.95</td>
<td>1.23</td>
<td>3.44</td>
<td>3.82</td>
<td>0.13</td>
</tr>
<tr>
<td><strong>PA</strong></td>
<td>1.20</td>
<td>0.31</td>
<td>1.07</td>
<td>1.49</td>
<td>4.79</td>
<td>0.43</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Much of the focus on pathogenesis of LAP has been on altered neutrophil function suggesting that inefficient chemotaxis, poor activation, or failure to resolve inflammation prevent an appropriate response to microflora and promote tissue destruction.
Biofilm state not biofilm source

• Biofilm source (i.e. derived from a healthy or diseased site) does not influence pattern of immune response,

• Biofilm state (i.e. homeostatic vs. disequilibrium) does influence the pattern of immune response
Same biofilm different response

Intact biofilms are less recognizable and more difficult to phagocytose by macrophages due to the extracellular matrix that coats and ‘hides’ the bacteria in the biofilm.

Differences in the responses to intact versus dispersed biofilms in both the magnitude and the pattern of the cytokine response,

Macrophages are skewed to an M2 phenotype when exposed to S. aureus in a biofilm, rendering them less inflammatory and less capable of clearing the biofilm than M1-type macrophages.

Does the host select the bacteria

- E. coli (×)
- S. aureus (◆) (◊)
- A. baumannii (−)
- P. aeruginosa (●), (○), (∗)

Gonzalez  mSphere 2016 Apr 27;1(2).
Conclusion

• Dynamics not constituents of biofilm may determine outcome
• Window of opportunity for bacteria to have any influence on ulcers which is over by 24 weeks.
• Black box bacteriology v stamp collecting
• Antibiotics treat infection but will not heal wounds and may have unexpected effects on biofilm.
So should we treat the Pseudomonas?

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- Method: Stokes
- Erythro/Clarithromycin: Susceptible
- Flucloxacillin: Susceptible

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*** END OF REPORT ***
Thanks

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