Intra-osteoblastic persistence of methicillin-susceptible Staphylococcus aureus: An adaptive mechanism of bone and joint infections chronicity

F. Valour, J.P. Rasigade, S. Trouillet, J. Gagnaire, A. Bouaziz, M. Bès, H. Meugnier, S. Lustig, F. Vandenesch, T. Ferry, F. Laurent
INSERM U851, Hospices Civils de Lyon, France – florent.valour@chu-lyon.fr

**INTRODUCTION**

Bacterial invasion of non-phagocytic cells contributes to the pathogenesis of bone and joint infections (BJI). Notably, community-acquired methicillin-resistant *S. aureus* (MRSA), responsible for acute BJIs, are cytotoxic but have a low persistence rate within osteoblasts. On the contrary, hospital-acquired MRSA can persist in bone cells with a low cytotoxicity rate, leading to chronic and indolent BJI. The interaction of methicillin-susceptible *S. aureus* (MSSA), the leading cause of BJI, with bone cells has not been studied because of its high genetic diversity.

We assessed bone cell invasion and cytotoxicity induced by BJI MSSA clinical isolates in an *ex vivo* model of intracellular infection. Genetic background of the isolates and the functionality of accessory gene regulator (*agr*), the main staphylococcal virulence factor regulator, were evaluated.

**METHODS**

- **Patient selection:** all cases of MSSA BJI included in the databases of the infectious diseases unit and bacteriology laboratory of our institution between 2001 and 2011.
- **Clinical data and strain collections:** Retrospective collection from medical records using a standardized case report form. Corresponding strains were extracted from the collection of the bacteriology laboratory, storing all samples at -80°C since 2001.
- **Definitions:** MSSA BJI diagnosis was based upon the presence of clinical evidence of infection and at least one deep bacteriological sample or a blood culture positive for MSSA. BJI were considered to be acute if delay from symptoms onset to bacteriological diagnosis was ≤ 4 weeks.
- **Bacterial interaction with bone cells:** Infection of MG63 human osteoblasts in a gentamicin protection assay assessing bacterial internalization and cytotoxicity at 24h, with the laboratory strain 8325-4 in each experience as a control.

**RESULTS**

- **Studied population:** 211 patients identified, 9 excluded (6 missing clinical data, 3 diabetic foot infections). 116 corresponding clinical strains identified, 21 excluded (19 collected at time of relapse, 2 stable small colony variants). 95 patients with their corresponding strains were included.

<table>
<thead>
<tr>
<th></th>
<th>Acute BJIs</th>
<th>Chronic BJIs</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>56</td>
<td>39</td>
<td>21</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53±20</td>
<td>56±19</td>
<td>49±20</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>60/35</td>
<td>38/26</td>
<td>22/29</td>
</tr>
<tr>
<td>Orthopaedic device</td>
<td>84 (67.4%)</td>
<td>47 (73.4%)</td>
<td>11 (14.8%)</td>
</tr>
<tr>
<td>Osteosynthesis</td>
<td>17</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Prosthesis</td>
<td>9</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Delay symptom - diagnosis (w)</td>
<td>8±1.4</td>
<td>11±1</td>
<td>23±1.5</td>
</tr>
</tbody>
</table>

- **Interaction with human osteoblasts:**
  - Internalization rate of strains isolated from chronic BJIs (169% of 8325-4) was higher than for acute BJIs (158%) (p=0.047)

  ![Internalization rate](image)

  - Highly significant correlation between internalization rate and BJIs evolution delay (Spearman coefficient 0.25, p=0.015)

  ![Evolution delay](image)

  - No difference was shown regarding cytotoxicity

  ![Cytotoxicity](image)

- **Genetic diversity:** spa-type could have been obtained for 88 strains, revealing 90 different spa-type, grouped in 10 clusters.

- **agr functionality:** 15 out 88 tested strains were found to have non-functional agr locus (17%). Persistence rate of these D- isolates (337±212%) was significantly higher than D+ ones (169±144%, p=0.0003) and BJI evolution delay trends to be higher in D- strains (p=0.064).

**CONCLUSIONS**

Our results provide the first demonstration of the correlation between MSSA intra-osteoblastic persistence and BJI chronicity. This mechanism, which appears to be independent from genetic background and can be observed after *agr* dysfunction, could be an adaptative pathophysiological pathway of the transition to chronicity of BJI.