Added diagnostic value and impact on antimicrobial therapy of 16S rRNA PCR and amplicon sequencing on resected heart valves in infective endocarditis

B. Peeters*, P. Herijgers+, J. Verhaegen+, K. Beuselinck+, W. E. Peertmans*, M.-C. Herregods§, S. Desmet, K. Lagrou

*University Hospitals of Leuven, Laboratory Medicine, Leuven, Belgium; †University Hospitals of Leuven, Cardiothoracic Surgery, Leuven, Belgium; §University Hospitals of Leuven, Internal Medicine, Leuven, Belgium; ‡University Hospitals of Leuven, Cardiology, Leuven, Belgium, Abstract Reference No: 3121

Background
- Identification of the causative agent in infective endocarditis (IE) is crucial for adequate therapy.
- Blood cultures are negative in 2-30% of infective endocarditis patients and heart valve culture is insensitive (sensitivity 24-39%) [1].
- 16S rRNA PCR and amplicon sequencing (16S rRNA PCR) performed on IE heart valves proved to be accurate and sensitive but data on diagnostic performance and impact on antimicrobial therapy are scarce [2].

Materials and Methods
- All patients undergoing valve surgery at the University Hospitals of Leuven for definite or possible IE, according to modified Duke Criteria, were prospectively included over a 3-year (2013-2016) period.
- 16S rRNA PCR (UMD-Tissue Molzym, Bremen Germany) was performed on all valves.
- From each included patient at least 2 blood culture sets were collected. Valves were cultured in TSB medium for 7 days with subculturing if growth was observed.
- Coxiiella burnetii serology was performed in 2 cases of culture negative infective endocarditis.
- Criteria were used, defining the microbial cause of infective endocarditis [1].

Results
- 127 patients included, pre-operatively classified as definite (n=115) or possible (n=12) IE.
- Added diagnostic value and clinical impact of valve 16S rRNA PCR is shown in Table 1. Results of diagnostics tests in 120 post-operative definite IE patients are shown in Table 2.
- Sensitivity of BC (87%) and 16S rRNA PCR (87%) was higher (p ≤ 0.05) than of VC (25%) in patients (n=120), post-operatively classified as definite IE. The remaining patients (n=7) were post-operatively classified as rejected IE.
- Valve origin, the causative pathogen or effective pre-operative antibiotics did not influence sensitivity of 16S rRNA PCR.
- Sensitivity of BC (53%), 16S rRNA PCR (82%) and VC (41%) in patients (n=17) with post-operative definite IE, not receiving antibiotics prior to valve surgery was not different (p > 0.05).

Conclusions
- Valve 16S rRNA PCR was significantly more sensitive than VC and confirmed positive BC results in 66% of cases.
- In 21% of cases, 16S rRNA PCR clarified culture results or was the only method detecting and identifying the causative pathogen.
- In 10% of cases, molecular testing results influenced antimicrobial therapy.
- The very good performance characteristics and clinical impact of molecular testing of heart valves supports the incorporation of molecular testing in diagnostic criteria for IE.

Table 1: The added diagnostic value and clinical impact of valve 16S rRNA PCR.

<table>
<thead>
<tr>
<th>Causative pathogen detected only by 16S rRNA PCR</th>
<th>Added diagnostic value N=127 (%)</th>
<th>Impact on antimicrobial therapy N=127 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification of the causative pathogen in cases with mixed or discordant BC/VC results</td>
<td>9 (7)†</td>
<td>6 (5) ‡</td>
</tr>
<tr>
<td>Negative result in culture-negative pre-operative definite/possible IE cases, all classified as rejected IE post-operatively</td>
<td>7 (5)</td>
<td>5 (4) §</td>
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</tbody>
</table>

No added diagnostic value:
- Confirmation positive BC results: 84 (66)
- False negative result: 14 (11)
- BC positive for P. aeruginosa, VC positive for P. aeruginosa/S. sanguinis and 16S rRNA PCR positive for E. faecalis/Acinetobacter spp.: 1 (1)
- BC/VC positive for E. faecalis with positive but unidentifiable molecular result: 1 (1)

* Coxiiella burnetii (n=0), P. acnes (n=2), Coagulase-negative staphylococci (n=2), S. gallolyticus (n=1), S. mitis (n=1), A. actinomycescomitans (n=1), E. hogen (started after 16S rRNA PCR was positive for C. burnetii); ** Narrowing antimicrobial spectrum; ***Stop antibiotics.

Table 2: Results of diagnostic tests conducted in 120 post-operative definite infective endocarditis patients.

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