

COMBINATION OF NEXT GENERATION SEQUENCING AND CULTURE IMPROVE MICROBIOLOGICAL DIAGNOSIS OF PROSTHETIC JOINT INFECTIONS

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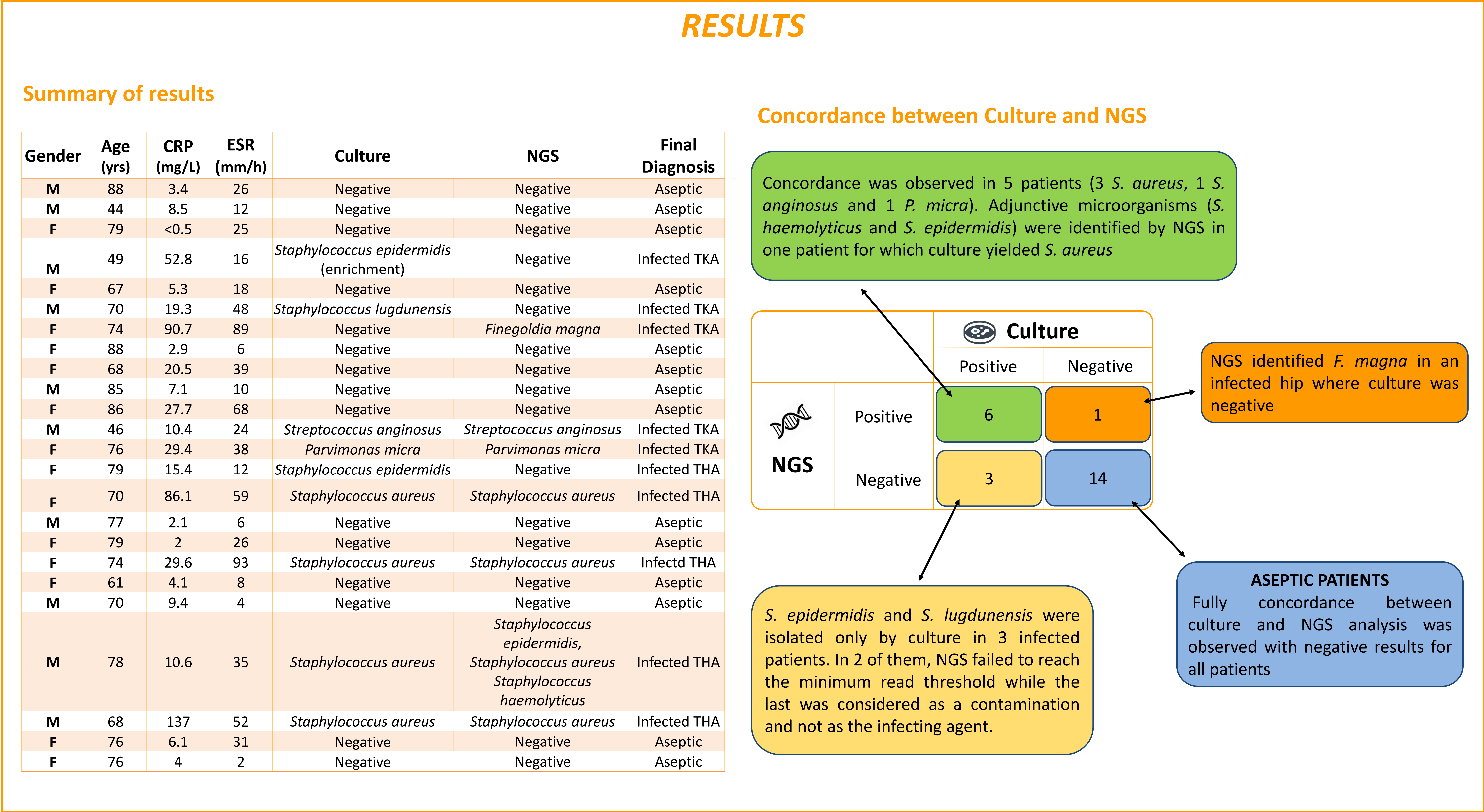
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BACKGROUND

- Infection is a significant and challenging complication of total hip and knee arthroplasties.
- Identification of the causative pathogen remains a crucial challenge in diagnosis of prosthetic joint infections (PJIs).
- Culture remains the gold standard in the diagnostic workflow but some limitations, such as slow turnaround time and limited sensitivity, have been reported (1).
- Preoperative antibiotic treatment, presence of biofilm or of difficult to growth bacteria are the main causes for false negative results.
- To improve microbiological diagnosis, various molecular biology techniques have been developed in recent years to overcome the limitations of traditional culture methods (2).
- Recently next generation sequencing has been proposed as an important approach to ameliorate sensitivity of microbiological diagnosis of PJI (3).
- In this study we have evaluated the impact of NGS on diagnosis and management of PJI in a referral orthopaedic centre.

METHODS

- Samples from 25 patients with diagnosis of suspected PJI, aseptic implant failure or undergoing spacer removal in second stage revision were sent to Laboratory for culture (periprosthetic tissues, prosthetic implants or synovial fluid) and for NGS analysis (synovial fluid and tissues).
- Culture: tissues and implants were treated with 0.1% dithiothreitol before inoculation onto agar plates and into enrichment broths for aerobes and anaerobes, which were incubated for 15 days (4). Synovial fluids were centrifuged and the resulting pellet was plated as described above. Enrichment broths were checked daily and, when turbidity was observed, an aliquot was plated onto blood agar or Schaedler agar. Grown colonies were identified by use of biochemical assays.
- Samples for NGS analysis were stored at -80°C and sent to an External Service Laboratory (5). Sequencing targeted the V1 and V2 regions of bacterial 16S rRNA gene and the fungal transcribed spacer (ITS) regions. Microorganisms with a relative abundance above 2% were considered as clinically significant.



CONCLUSIONS

- Results indicate as combining culture with NGS could ameliorate sensitivity of microbiological diagnosis of PJI, thus optimizing patients management.
- Accurate sampling in surgery room and treatment of tissues and prosthetic implants with dithiothreitol has proven to be an effective strategy, since culture showed a sensitivity comparable to that of NGS.
- NGS may enable optimization of patient treatment, especially when difficult to growth microorganisms are involved in PJI or in case of previous prolonged antibiotic therapy. However, NGS results should be interpreted with caution, taking into account the patient’s clinical condition and, in case of negative NGS, it is advisable to wait for culture results.

References

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