The Potential of Metagenomic DNA Sequencing for Pathogen Identification in Orthopaedic Nonunion

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Purpose: Fracture nonunion remains a devastating complication that confers significant morbidity. While it is known that nonunions may occur for several reasons, broadly classified as septic or aseptic, the true extent to which the presence of microbes preclude fracture healing remains unknown. With the increasing availability of metagenomic sequencing techniques, such as next-generation sequencing (NGS), rapid and high throughput detection of all microbial DNA present within a clinical sample is now possible. It has therefore been proposed that a significant percentage of nonunions actually harbor microbes that escape detection by conventional culture methods. To date, no study has examined the metagenomic profile of fracture nonunions or explore the clinical relevance of this signal. Our aim was to investigate the role of NGS in the diagnosis of nonunion compared to culture, as well as its association with treatment outcomes in terms of fracture union.

Methods: In this prospective study, samples were collected from 20 consecutive patients undergoing open surgical intervention for long bone nonunion (7 femurs, 9 tibias, 4 humeri). Nonunion was defined as a failure to progress towards union within an anticipated time frame as defined by the attending surgeon. We excluded patients with pathological fractures or those on preceding antibiotic therapy. Three tissue samples (superficial membrane; proximal and distal fracture) and 3 intraoperative swabs (fracture site; hardware) were obtained at the time of the surgical procedure and sent for NGS. Tissue specimens from concordant sites were sent to the institutional laboratory for culture. Patients were followed for a minimum of 6 months (range, 6-11) for radiological evidence of union. Concordance and bivariate statistics were used to compare NGS, culture, and union rates. Principal coordinates analysis of NGS species diversity was also conducted.

Results: Among the total cohort, 14 nonunions were culture-negative (14/20; 70.0%) and 6 were culture- positive (6/6; 100.0%). Among the positive culture cases, complete concordance between NGS and culture results was noticed in 6 cases (100% dominant species similarity). Among the 14 cases of culture-negative nonunion, NGS identified a microbe in 6 cases (42.9%). NGS detected multiple organisms in most positive samples (mean 2.9 microbes) but 1 organism was typically dominant. Of note, positive NGS signal in culture- negative cases was inversely associated with fracture union at interim follow-up (50% vs 75%); however, this trend did not reach statistical significance ($P = 0.12$).

Conclusion: NGS may be a useful adjunct in identification of the causative organism in culture-negative nonunion. Our findings suggest that some cases of nonunion may have additional organisms that escape detection when culture is used. Further multicenter work is required to determine the clinical implications of organisms detected on metagenomic sequencing.

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