QUESTION 13: Do bacteriophages have a role in treating multidrug-resistant PJI?

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Response:

Unknown. Although some preclinical and clinical studies have demonstrated a good safety profile as well as promising therapeutic effects using bacteriophages for treating bone and joint infections, further clinical research using bacteriophage therapy in patients with multidrug-resistant PJI is required.

There are known obstacles to bacteriophage therapy, including the fact that bacteriophages are neutralized in serum and relevant pathogens contain CRISPR/cas9 immunity against bacteriophage. Phages are usually bacterial strain specific; thus, a cocktail of different bacteriophage lineages may be necessary to effectively treat biofilm-mediated infections.

Level of Evidence: Consensus

Delegate Vote: Agree: 100%, Disagree: 0%, Abstain: 0% (Unanimous, Strongest Consensus)

Post Meeting Rationale:

Authors performed the following two searches 1: (bacteriophages [mesh] OR Phage Therapy [mesh]) AND (osteomyelitis [mesh] OR bone diseases, infectious [mesh] OR Prosthesis-Related Infections [mesh]) 2: (bacteriophages* OR Phage Therap*) AND (osteomyelitis* OR bone diseases* OR Prosthesis Related Infections* OR prosthetic joint infection*). Searches were performed on Medline from inception till February 2018.

Novel treatment strategies focusing on disrupting biofilms are being developed [1]. Utilization of lytic bacteriophages to eradicate bacteria causing biofilms is one of the promising emerging technologies [2,3].

Bacteriophages are natural viruses that infect bacteria. They are one of the most abundant organisms in the biosphere. Each bacteriophage is specific to a particular microbial species. Like all viruses, phages are only able to replicate inside their host cells. Lytic phages inject their genetic material into the host bacterial cell, cause bacterial cell lysis that liberates subsequent new phage particles. These new particles allow successive infection of additional bacteria in a rapid and exponential pattern, facilitating the complete eradication of the bacteria. By their nature, bacteriophages are good candidates for antibacterial therapy. Indeed, they target specifically a bacterium, as long as the corresponding host bacteria is present. In comparison with antibiotics, this phenomenon is self-sustained and exponential but may take few administrations to achieve. Moreover, lytic bacteriophages do not affect eukaryotic cells and not impact the gut microbiota when administered locally.

Bacteriophage technology is of interest in patients with multidrug-resistant PJI as: (i) multidrug-resistant PJI are becoming more and more frequent; [4,5] (ii) the rate of relapse is high in patients with PJI caused by multidrug-resistant pathogen; [5–7] (iii) bacteriophages and antibmicrobials are synergistic; [8,9] (iv) there is no cross-resistance between antimicrobial resistance and bacteriophage efficacy; [2,4–8,10] (v) bacteriophages may have anti-biofilm activity in some *in vitro* studies and animal models; [2,9,11] and (vi) recent human and animal trials using phage therapy have not found any local tissue toxicity or any adverse effects to the host [12–17].

Few case series have been published in the literature, including patients with pyogenic native joint infection, chronic osteomyelitis, suppuration after bone fracture and diabetic foot osteomyelitis [18–22]. In preclinical studies using animal models for PJI bacteriophages were found to prevent bacterial adhesion and also effectively disrupt the formation of biofilm [9,23]. Animal studies also have proven synergism between antibiotics and bacteriophages [9]. In another animal study, Kishor et al. [22] studied the efficacy of several phages used in conjunction as a treatment modality for chronic osteomyelitis caused by MRSA in rabbits. The study showed that the combination of specific phages selected based on their virulence against various clinical MRSA strains was effective in eradicating the infection, thus suggesting that a "tailor-made cocktail" of phages can alone be effective in targeting specific bacteria in the setting of a chronic infection.

No case series including patients with prosthetic joint infection has been published (we retrieved only 2 cases from a French series of bone and joint infection treated with bacteriophages) [2]. In the Georgian practice, specific phages mixtures are used, such as the "pyophage" cocktail that contains phages against *S. aureus*, *Streptococcus*, *Proteus*, *P. aeruginosa* and *E. Coli* or specific bacteriophages targeting specifically staphylococci, as the Sb-1 phage (that could be imported in the USA), the bacteriophage K or the bacteriophage ISP [18]. In Poland, phage(s) are selected from a bank based on their activity on the patient's strain to adapt the treatment (personal medicine) and to ensure antibacterial activity of phages used [19,20]. All these bacteriophages are classically prepared with a bacterial inoculum, *in vitro* infection with the bacteriophage, and purification of the preparation in aliquots at 10^7 to 10^8 PFU/mL. These preparations are approved by local authorities, but do not respect European "good manufacturing practice" (GMP) standards for conducting clinical trials and targeting Market Authorizations (MA). Indeed, the final product requires total elimination of bacterial components that are generated during the production process such as toxins, in order to limit pyrogenicity and adverse events that may arise during phage administration/use, especially when the phage is administered intravenously or directly in a joint cavity. As a consequence, bacteriophages are currently not injected directly into the joint in patients with PJI, but locally throughout the fistula and/or orally in patients with chronic osteomyelitis [19–21].

Recently, an European multicentric clinical trial evaluating phage therapy of burn wound infections has been done using *P. aeruginosa* and *E. coli* bacteriophages from a GMP French bioproduction process that was implemented according to European Medicine Agency standards (ClinicalTrials.gov Identifier: NCT02116010). The French team from the Lyon BJI study group (also called CRIOAc Lyon, a regional reference center for the treatment of complex bone and joint infection in France; http://www.crioac-lyon.fr) has treated as salvage therapy, under the supervision of the French health authorities, 3 patients with chronic bone and joint infection (1 osteomyelitis due to extensively drug-resistant *P. aeruginosa*; and 2 *S. aureus* PJI) with bacteriophages that follows the same process of production. For all the patients, the cocktail was

personalized and selected based on the bacteriophage susceptibility of the clinical isolates (phagogram; similar principle as antibiogram, but with bacteriophages) that was isolated after a joint puncture before the surgery. The two patients with PJI had chronic infection with purulent discharge and were treated with DAIR supplemented with a direct administration of the bacteriophage *S. aureus* cocktail in the joint cavity at the end of the procedure. Both patients are doing well during the follow-up of 12 and three months, respectively (unpublished data). A randomized clinical trial called PHAGOS will start soon in France, to evaluate the addition of *S. aureus* bacteriophage in patients with relapsing *S. aureus* PJI. The availability of *P. aeruginosa*, *E. coli* and *S. aureus* with GMP standard in France is a great opportunity to evaluate the phage therapy as an additive treatment in patients with PJI, especially in patients with multidrug-resistant PJI.

Questions remain related to treatment by phage such as timing, duration, methods of delivery, route of administration and immunogenicity. Limitations of present studies include the reduced spectrum of bacteria studied, which are limited to MRSA and *P. aeruginosa*, without considering coagulase-negative staphylococci (CoNS), which substantially contribute to PJI [24]. In addition to these there is a concern with regards to the immunogenicity of phages and resulting diminished therapeutic efficacy [25]. Moreover, the Workgroup identified several obstacles that seriously challenge the scientific premise of phage therapy for MSK infections including: 1) phages are neutralized in human serum although this may depend on the route of the phage therapy and requires more evaluations and effect on efficacy [26], 2) phages are strain specific leading to the need for a cocktail of phages to cover all possible bacteria in the biofilm, and 3) CRISPR Cas9 immunity engenders most bacterial pathogens evolutionary resistance to phage [27], and the bacteriophage therapy is not available in all countries including the USA and the UK. However, although phage treatment looks promising, appears to be cost effective and safe, further research including in vivo animal studies, is needed to identify parameters for clinical trials.

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