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EDITORIAL



# Towards clinical application of non-invasive imaging to detect bacterial infections

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**ABSTRACT**

In vivo imaging technologies offer a great potential for the diagnosis of difficult-to-treat bacterial infections. A major limitation of conventional imaging modalities is the lack of specificity to distinguish the site of bacterial infection from sterile inflammation. Targeted approaches like antibiotics linked to imaging tracers for detection of various bacterial pathogens or species-specific antibodies combined with anatomical imaging modalities are currently being evaluated to overcome this problem. Considering the recent progress in optical and targeted imaging that may accelerate preclinical development programs, clinical implementation of in vivo imaging modalities to detect bacterial infection foci becomes realistic in the future.

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The diagnosis of invasive and biomaterial-associated infections in humans is often difficult leading to a high rate of treatment failure. A major problem in in this regard is the lack of a sensitive, specific, non-invasive modality to detect bacteria during early stages of infection, when treatment is most effective. Currently, only indirect imaging modalities are in clinical use, as exemplified by fluorodeoxyglucose (FDG) positron emission tomography (PET), which visualizes increased glucose uptake by immune cells [1,2]. Alternatively, sites of inflammation are identified either by anatomical imaging or after injection of radiolabeled leucocytes and subsequent scanning [3]. Since these approaches do not discriminate between infection and inflammation, diagnosis often remains inconclusive. Therefore, imaging tools that directly target the invasive bacteria are highly desirable. Optical imaging and targeted clinical fluorescence imaging (FLI) in particular constitute a novel diagnostic approach that has attracted increasing attention in both preclinical and clinical applications [4,5]. FLI relies on the administration of an exogenous fluorophore that is excited by photons and emits light at a higher wavelength, which is then detected by a sensitive CCD camera. The obvious advantages are: i) non-invasive real-time imaging, ii) high resolution, iii) absence of radiation-related risks, and iv) relatively low costs. For example, targeted FLI for the detection of bacterial infections was performed by Panizzi et al., who described the use of a fluorescent prothrombin analog to trace *Staphylococcus aureus* endocarditis by targeting blood coagulation [6]. Sensitive, high-resolution imaging was achieved, suggesting possible clinical applications. However, the fluorescent prothrombin tracer targets a phenomenon caused by the bacteria (i.e., coagulation) rather than the bacteria themselves. Recently, van Oosten and colleagues have used fluorescently labelled vancomycin to specifically target and detect infections caused by *S. aureus* [7]. In this approach, the near-infrared fluorescence dye IRDye800CW was coupled to the antibiotic vancomycin. The *in vivo* applicability of vanco-800CW was tested in immunocompetent mice with myositis in the hind limb induced by intramuscular injection of engineered luciferase expressing *S. aureus*. Vancomycin directly binds to the cell wall of Gram-positive bacteria, and the clinically approved IRDye800CW allows near-infrared fluorescent optical imaging with high signal-to-noise ratios due to marginal auto-fluorescence and optimal tissue penetration. In the mouse myositis model, it was possible to discriminate bacterial infection from sterile inflammation *in vivo*. In addition, the *S. aureus* strain's constitutive bioluminescence facilitates localization of live bacteria by simultaneous imaging of the fluorescence signal derived from vanco-800CW and luciferase bioluminescence [7]. However, with this approach it is not possible to discriminate between different bacterial species as vancomycin binds to the cell wall of almost all Gram-positive bacteria. To detect specifically infections due to *S. aureus*, Pastrana and colleagues published in a recent issue of *Virulence* the use of a highly

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specific antibody for detection of *S. aureus* infectious foci by two different modalities, optical fluorescent imaging and PET imaging [8]. The authors selected an antibody that recognizes the broadly expressed surface antigen IsaA of *S. aureus* [9] in order to ensure detection of all clinical strains of the pathogen and coupled it to the near infrared dye 800CW. The antibody-fluorophore conjugate was evaluated in a human post-mortem implant model where *S. aureus* bacteria were placed under the skin, and in two mouse models. In order to test the antibody-800CW conjugate for specificity, the authors compared binding to wildtype bacteria with mutants lacking IsaA or the IgG-binding *S. aureus* proteins Spa, and Sbi, respectively. In all models, the detected signal was specific for IsaA, supporting the idea of using antibodies for *in vivo* imaging diagnosis of bacterial infections [8]. Furthermore, fluorescence imaging results correlated roughly with bioluminescence signals of luciferase (*lux*)-expressing bacteria. Taken together, this implicates high specificity of the anti-IsaA-800CW conjugate. Nevertheless, one limitation of optical imaging using fluorescence tracers is that only superficial infections can be visualized. To overcome this limitation and to visualize deeper seated infections, the authors used the antibody for PET imaging by labeling the anti-IsaA antibody with $^{89}$Zr. By this approach, a specific signal could be visualized up to three days after administration confirming the principal applicability of anti-IsaA antibody-800CW conjugate-derived detection of *S. aureus* infectious foci. Thus, immuno-PET imaging may be a promising approach to detect bacterial infections due to the high sensitivity of PET tracers combined with high specificity of monoclonal antibodies. A clear advantage of immuno-PET imaging is the ability to identify the etiological agent of an infection. This is, in contrast, not possible with a metabolism-sensitive tracer like [$^{18}$F] FDG that is not able to distinguish between inflammation, pathogen-induced infection, or sterile irritation. The best diagnostic results may be achieved when immuno-PET imaging is combined with computer tomography (CT) or magnetic resonance imaging (MRI) since the latter methods provide additionally information on anatomical structures [10]. Although radioactive labels should be restrictively used, application of low radioactive doses combined with high resolution offers a great potential for non-invasive clinical diagnostics of infections.

Recently, MRI-based imaging also has been applied to visualize infections due to *S. aureus* in an osteomyelitis [11], endocarditis [12], and soft tissue infection model [13,14]. Generally, these models detect inflammation initialized by immune cells as response of infected tissues to the infection process rather than visualizing directly the causative agent of an infection. Although direct visualization of bacteria has been achieved by use of iron-particle labelled *S. aureus* [15], for pathogen-specific diagnostics a targeted approach e.g. coupling of iron-containing nanoparticles with an antibody would be necessary. Nevertheless, MRI possesses a high potential when combined with other imaging modalities such as PET imaging as already mentioned before. Moreover, optoacustic imaging that has a higher penetration through tissues recently has coming up as a promising imaging modality. This technique is based on the photoacoustic effect providing functional information on tissue morphologies. Importantly, optoacustic imaging allows visualization of tissue changes up to 8 cm in depth compared to fluorescence-based optical methods reaching tissue penetration of less than 1 cm. This modality has been applied in preclinical models and in patients to evaluate its feasibility to increase the diagnostic sensitivity in cancer patients [16,17]. In principle, it can also be implemented in detection of infectious agents e.g. by coupling antibodies to an optoacustic tracer. However, further research is needed to evaluate the potential of optoacustic imaging for detection of bacterial or other infections and in particular its clinical benefit.

Overall, fast and accurate diagnosis of bacterial infections is fundamental to initialize specific and efficient treatment. Progress in targeted non-invasive imaging systems such as developed by Pastana and colleagues allow now the use of specific optical imaging methods to detect superficial infections. Moreover, PET imaging is suitable for visualization of deep-seated infections. However, all these methods are still under investigation and not available for routine diagnostics due to relatively high costs, limited availability of technical equipment, and the lack of standard procedures to select patients who would have a clear benefit from application of modern non-invasive imaging methods. In addition, all imaging methods described so far cannot discriminate between antibiotic sensitive or resistant strains. Therefore, a combination of molecular techniques to detect the bacterial species and the resistance properties e.g. by next-generation sequencing, coupled with non-invasive imaging techniques to localize exactly the site of an infection, especially for chronic or recurrent infections, may have the greatest potential to increase the power of clinical diagnostics towards application of personalized medicine in the near future.

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No potential conflicts of interest were disclosed.

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