Re-evaluating the Role of Antibodies in Tuberculosis

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Abstract: Tuberculosis (TB) is a disease found in every country on Earth. About a quarter of the global population is estimated to be infected with Mycobacterium tuberculosis, the causative agent of TB, with over 10 million new TB cases reported annually. Currently, TB ranks as the second leading infectious killer after COVID-19. Research to develop novel interventions against TB represents a global health priority. TB research over the last several years focused on cellular immune responses, while the humoral response was largely neglected. This mini-review discusses evidence supporting a protective role of antibodies in TB, and a potential role of antibodies in TB vaccines and diagnosis.

Keywords: Tuberculosis, *Mycobacterium tuberculosis*, antibody, TB diagnosis, TB vaccine

Introduction

Tuberculosis (TB), caused by the bacillus *Mycobacterium tuberculosis* (*Mtb*), is a serious global health issue. An estimated number of 1.6 million people die from TB annually (World Health Organization, 2022). The Covid-19 pandemic, and with it the disruption of health care and TB screening programs, had damaging effects on the global TB burden. For the first time since 2019, a rise in deaths from TB was recorded, while a large global fall in newly diagnosed cases were reported. As a consequence, more community transmission and a surge in new TB cases are expected in the years to come (World Health Organization, 2022).

Decades of research have tremendously advanced our understanding of TB. Yet, the TB epidemic is sustained by an insufficient understanding of underlying immune mechanisms. Global control can only be achieved by a combined effort to improve diagnostics, treatment, and novel vaccines. Our knowledge in the TB field is constantly evolving, unraveling the complexity of the host-pathogen interaction between *Mtb* and the immune system. Johann Wolfgang von Goethe once said: "We know accurately only when we know little, with knowledge doubt increases." In science, knowledge is accumulated through systematic observations and discoveries. The certainty of observations and findings can only be obtained by repeated confirmations and re-evaluations. As molecular and immunological methods advance, so does our knowledge. Thus, we constantly need to re-evaluate the solidity of current beliefs and in some instances, corrections of previous paradigms are required.

Why antibody responses were neglected in TB

In the early 20th century, serum therapy became a clinical success in treating multiple viral and bacterial diseases such as measles, polio, pneumococcus, Haemophilus influenza B, and meningococcus (Casadevall & Scharff, 1995). Conversely, the effects of serum therapy in TB patients were conflicting. Several studies claimed an improvement of conditions and even cure of TB patients with serum therapy, while others did not observe any beneficial effects after serum therapy (Glatman-freedman & Schweinitz, 1998). Serum transfer experiments in animals either did not provide clarification on the matter (Glatman-freedman & Schweinitz, 1998). In contrast, several experiments showed that the

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transfer of lymphoid cells from immunized animals into non-immunized animals provided protection (Steigler et al., 2019).

At the time, a paradigm of labor division evolved, stipulating compartmentalization of the immune defense against intracellular and extracellular pathogens. Antibody responses were believed to combat extracellular pathogens, while cellular responses defend pathogens in the intracellular space. Based on inconsistent results in serum therapy, successes in cellular passive transfer experiments, and a new paradigm of labor division in the immune system, TB research began to concentrate on cellular immune responses while humoral responses faded into obscurity. Nowadays, it has been long recognized, that the immune system is a complex interactive system in which cellular and humoral components act in concert with one another to provide an effective and powerful defense.

This review will discuss current evidence of the functional role of *Mtb*-specific antibodies to protect against TB and their potential utility in TB vaccines and diagnosis.

Functional antibodies in TB

The functional role of antibodies in *Mtb* infection remains unclear. Several lines of evidence suggest the biological activity of antibody responses in TB. Healthcare workers (HCWs), who had an occupational risk of *Mtb* exposure, produced protective antibodies. However, functional *Mtb*-specific antibodies were not found in all HCWs. This study highlights the heterogeneity of *Mtb*-specific humoral immune response. Sera from donors with protective antibody responses decreased the growth of *Mtb* bacilli in a whole-blood assay in comparison to sera from donors having non-protective antibody responses. These effects were dose-dependent, with higher antibody doses improving antibody-mediated protection (Li et al., 2017).

A recent study utilized monoclonal antibody technology to investigate the functionality of antibodies induced by *Mtb* infection. In this study, memory B cells were isolated from a recovered TB patient to generate distinct *Mtb*-specific monoclonal antibodies. *Mtb* infection elicited antibodies specific for the 38-kDa phosphate binding periplasmatic protein PstS1, which is part of the *Mtb* phosphate-specific transporter (Pst) (Watson et al., 2021). PstS1-specific antibodies were capable of inhibiting the growth of *Mtb* in a human whole-blood mycobacterial growth inhibition assay (MGIA). These results were confirmed in *in vivo* studies. Mice that received PstS1-specific antibodies (intraperitoneal injections) had lower lung bacterial burden than control mice two weeks post *Mtb* infection.

Antibodies have a large range of functions. Antibodies either directly combat pathogens or activate immune cells to mediate protection. The functionality of antibodies is dependent on isotype and subclass. The first isotype produced during primary infection is the pentameric Immunoglobulin (Ig)M. IgM is predominantly present in blood but can also be secreted across mucosal surfaces. The dominant isotype during a secondary infection is IgG. IgG is the most abundant isotype in blood and is further classified into subclass IgG1, IgG2, IgG3, and IgG4. The dominant isotype found in the mucosa is IgA in its dimeric form (IgA2). IgE and IgD participate in allergic reactions and B cell maturation, respectively (Murphy, 2012).

Neutralization may prevent *Mtb* infection of target cells. Antibody neutralization is commonly employed in vaccine strategies (Plotkin, 2010). Neutralizing antibodies could inhibit the infectivity of *Mtb* by directly binding to the surface of the pathogen and sterically hindering its attachment to host cells (Fig. 1). Developing TB vaccines that could disrupt entry into host cells inhibiting initial infection, could be a promising way to prevent the manifestation of TB in the first place.

The tertiary structure of antibodies can be functionally divided into two segments: An antigen binding part, the antigen binding fragment (Fab), and a constant region, the crystallizable fragment (Fc), which interacts with Fc-receptors (FcR) on immune cells. Neutralization is an Fc-independent mechanism and is solely dependent on the Fab region. A study by Hamasur et al. demonstrated that lipoarabinomannan (LAM, a cell wall component of *Mtb*)-specific Fab fragments prolonged the survival of

mice (Hamasur et al., 2004). This study indicates that neutralizing antibodies could prevent *Mtb* infection.

Activation of cell-mediated immune response may be of more importance in the defense against *Mtb* than simple neutralizing responses. Antibody-dependent cellular phagocytosis (ADCP) may be important in killing engulfed *Mtb* bacilli (Fig. 1). Opsonization is the targeting of pathogens with antibodies for the uptake into phagocytic cells. The crosslinking of surface FcR by antibodies bound to *Mtb*, activates phagocytic cells to kill internalized *Mtb* bacilli. Serum antibodies from naturally *Mtb*-infected, healthy participants in India enhanced phagocytosis and intracellular killing in phagocytic macrophage cells that were isolated from the same donor (Kumar et al., 2015). Opsonization could be required to sufficiently activate phagocytic cells to kill internalized *Mtb* bacilli. On the other hand, the mechanisms of opso-phagocytosis could also be exploited by *Mtb* to facilitate uptake into host cells. Zimmerman et al. observed an enhanced infection of a human lung epithelial cell line when incubating *Mtb* with human *Mtb*-reactive IgG antibodies prior to *in vitro* infection. In contrast, incubating *Mtb* with IgA of the same antigen-specificity reduced *Mtb* colony-forming units (CFU) (Zimmermann et al., 2016). Different antibody isotypes could have different roles in TB, and either be protective or detrimental in *Mtb* infection.

Mtb-infected cells could be destroyed by antibody-dependent cellular cytotoxicity (ADCC). Antibody-dependent crosslinking of the FcR activates cytotoxic cells, such as NKs or CD8 T cells, to release the cytotoxic molecules toward the target cell (Fig. 1). The cytotoxic molecules granzymes (Gr) and perforin (Perf) form holes in the outer membrane of the infected cells, ultimately killing the cell through lysis. PPD-specific IgG from latently *Mtb*-infected individuals, but not from patients with active TB, enhanced ADCC (Lu et al., 2016). The quality of antibody responses may be a crucial factor in dictating their protective ability.

Antibodies could support T cell responses in TB. T cells are crucial in protection against TB (Jasenosky et al., 2015). Immune complexes that are comprised of *Mtb* bacilli coated with *Mtb*-specific antibodies, could result in increased processing and antigen presentation to T cells by antigen-presenting cells (APCs) such as dendritic cells (DCs) or macrophages (Fig. 1). When DCs were pretreated with sera from participants vaccinated with the TB vaccine Bacillus Calmette-Guérin (BCG), increased proliferative ability of CD4 and CD8 T cells was seen in comparison to an assay using DCs that were infected with BCG pretreated with pre-vaccination sera from the same participants. BCG pretreated with post-vaccination sera further enhanced the production of interferon-gamma (IFN-γ), a key cytokine in the protection against TB, in CD4 and CD8 T cells; and CD8 T cell cytotoxic responses (CD107a) (de Vallière et al., 2005).

A role for antibodies in TB vaccines

The implementation of effective therapeutic and preventive TB vaccines is critical to archive control of the global TB epidemic. At present, 16 vaccine candidates are at different stages of clinical trials (World Health Organization, 2022). Despite antibodies being a protective correlate in most commercially available vaccines today, antibody functionality in TB vaccines remains unclear. Traditionally, TB vaccine candidates intend to induce robust cellular immune responses to control the replication of *Mtb* after infection. More recent clinical TB vaccine trials started to include the evaluation of vaccine-induced antibody responses in addition to determining the immunogenicity of T cell responses. TB vaccine-induced antibody responses could protect against the development of TB disease or even prevent initial infection.

It has been more than one hundred years since BCG was first administered and it is still the only approved TB vaccine today. Today, BCG is one of the most widely applied vaccines worldwide and is routinely given intradermally after birth in TB-endemic countries. Vaccination with BCG effectively confers protection from disseminated TB disease in children but fails to protect against the most common form of TB, pulmonary TB, in adolescents and adults (Trunz et al., 2006). Pulmonary TB is a major concern of transmission fueling the TB epidemic. Droplets containing as little as one bacillus can cause TB disease (Riley et al., 1995). To prevent person-to-person transmission novel vaccine candidates need to protect against pulmonary TB.

BCG vaccination induces mycobacteria-specific antibody responses. In BCG-vaccinated South African infants, antigen 85 complex A (Ag85A, a subunit of the mycoltransferase Ag85 in *Mtb*)-specific IgG correlated with a reduced risk of TB disease (Fletcher et al., 2016). This study did not investigate the functionality of Ag85A-specific IgG; thus, it is not unclear whether vaccine-induced antibodies are a mechanistic or non-mechanistic correlate of protection. Several *in vitro* studies were able to demonstrate a protective function of BCG-induced antibody responses. Post-BCG vaccination sera collected from participants in non-TB epidemic regions (UK and US) mediated enhanced macrophage phagocytosis and intracellular growth inhibition compared to pre-vaccination sera (Chen et al., 2016). In a similar experiment, de Vallière *et al.* demonstrated that growth inhibition by phagocytic neutrophils and monocytes/macrophages was abrogated when IgG antibodies were depleted from post-BCG vaccination sera (de Vallière et al., 2005).

The quality of the immune response may be sensitive to the route of immunization. Mucosal and systemic BCG vaccination had a profound impact on the ability of antibodies to protect against pulmonary TB. Rhesus macaques vaccinated with BCG over the pulmonary mucosal route (endobronchial instillation) had decreased lung *Mtb* CFU and pathology in comparison to the intradermally BCG-vaccinated group (Dijkman et al., 2019). The primary correlate of protection in mucosal vaccinated macaques was PPD-specific IgA in bronchoalveolar lavage (BAL). PPD-specific IgA levels in BAL were 1 log higher in mucosal vaccinated macaques than in the intradermally vaccinated group, pointing to the importance of inducing protective antibody responses at the site of infection. Another study induced robust BCG-specific IgM responses in plasma and BAL by vaccinating macaques intravenously with BCG. Mycobacteria-specific IgM titers in plasma and BAL negatively correlated with *Mtb* burden in lungs (Irvine et al., 2021). LAM-specific antibodies from intravenously vaccinated macaques induced significantly (though moderately) higher antibody-dependent neutrophile phagocytosis (Irvine et al., 2021). Although endobronchial or intravenous vaccination may not be suitable routes for humans, these studies show that mycobacteria-specific antibodies can be protective and targeted by vaccine strategies.

TB vaccine candidates induce vaccine-specific antibody responses in clinical trials. The investigational TB vaccine candidate M72/AS01$_E$ is a subunit vaccine containing a recombinant fusion protein derived from two highly immunogenic *Mtb* antigens (*Mtb*32A/Rv0125 and *Mtb*39A/RV1196) and the liposome-based adjuvant AS01$_E$ (Penn-Nicholson et al., 2015). Two doses of M72/AS01$_E$ vaccine induced M72-specific IgG (Tait et al., 2019). Adults who received M72/AS01$_E$ vaccination had a 49.7% reduced rate of progressing to active TB disease compared to the placebo group. Robust mycobacteria-specific T cell and antibody responses were present in M72/AS01$_E$ vaccinated participants (Tait et al., 2019). It is unclear whether vaccine-induced antibody responses contributed to lower rates of TB progression, but M72-specific IgG responses were shown to be long-lasting and still detectable 3 years post-vaccination in HIV-positive and HIV-negative participants (Kumarasamy et al., 2018). Other subunit TB vaccine candidates, H4:IC31 (antigens: Ag85B and TB10.4) and H56:IC31(antigens: ESAT-6 and Rv2660c) were shown to induce anti-H4 and H56-IgG1 and IgG3 serum antibodies (Bekker et al., 2020). Vaccine-specific antibody (anti-Ag85 IgG) responses were also seen after vaccination with the viral vector vaccine candidate ChAdOx1 85A prime – MVA85A boost (Wilkie et al., 2020). The functional role of mycobacteria-specific antibodies induced by new TB vaccine candidates has yet to be investigated. Utilizing vaccine-induced antibody responses may be a crucial step in improving vaccine efficacy and changing the trajectory of the ongoing TB epidemic.

Antibodies as tools in TB diagnosis

Prompt detection of active TB cases and treatment initiation are mandatory to halt the global TB burden. During early stages, pulmonary TB patients have similar symptoms to those presenting with a common cold including cough, phlegm, and fever, which presents another hurdle in TB diagnosis. The majority of *Mtb*-infected individuals will remain asymptomatic, a state termed as latent TB (LTBI). It is postulated that only 5-10% of *Mtb*-infected individuals develop TB disease over a lifetime

(Vynnycky & Fine, 1997). A more recent study in the US suggests this number to be even lower with approximately 2% lifetime risk (Menzies et al., 2021). Despite many *Mtb*-infected individuals being asymptomatic, low transmission may still occur, presenting another challenge in halting the TB epidemic (McLean et al., 2019).

The diagnostic field in TB is constantly progressing to improve the detection of active TB cases. Yet a major drawback is the reliance on sputum samples in TB diagnosis. Microbiological diagnosis, including sputum smear microscopy and bacterial culture, detect the presence of *Mtb* bacilli in sputum. The staining of acid-fast bacilli in sputum smear microscopy is a fast, simple, and cost-effective tool that is employed in many under-resourced countries. However, a major disadvantage of smear microscopy is its low sensitivity. Sputum smear diagnosis is further limited by the inability to differentiate between *Mtb* and non-tuberculose mycobacteria or between live and dead bacilli (Desikan et al., 2017). Mycobacterial culture presents a much more sensitive but relatively costly tool. The high-risk nature of *Mtb*, requires the set-up of a high-containment laboratory. Due to the slow growth rate of *Mtb*, a long detection time is delaying culture-based diagnosis. A reliable, highly accurate alternative to microbiological detection is the molecular test GeneXpert, a nucleic acid amplification device that detects the DNA of *Mtb*. GeneXpert provides a speedy result and can even indicate drug resistance. The downside of GeneXpert is the high cost of purchasing the machine and test cartridges (Brown et al., 2021).

The World Health Organisation (WHO) calls for the development of non-sputum-based diagnostic tests as a key priority in the field of TB diagnostics (World Health Organization, 2021). Sputum samples can be difficult to obtain from children and HIV-co-infected individuals. In addition, the paucibacillary nature of TB diseases in children and HIV-co-infected individuals severely lowers the sensitivity of sputum-based diagnosis (Sabur et al., 2017; Venturini et al., 2014). A further concern is that the presence of bacilli in sputum equates to an advanced disease state, in which lung tissue destruction has taken place. Early diagnosis and treatment are imperative to prevent permanent disabilities due to progressed TB disease (Alene et al., 2021).

There is an urgent need to develop rapid, inexpensive, and easy-to-use diagnostic tests for TB. Urine is an easily accessible sample in all age groups. A non-invasive Urine point-of-care test was developed to detect the presence of the mycobacterial cell wall component LAM in urine. However, this test is restricted to HIV-positive individuals, and the vast majority of patients with active pulmonary TB are HIV-negative (Bulterys et al., 2019).

Blood is the most common specimen used in laboratory testing. Blood-based diagnostic assays for TB are the Interferon-gamma release assays (IGRAs) QuantiFERON Gold in-tube (QFT-GIT) test and the T-SPOT.TB assay. Both assays stimulate immune cells with *Mtb*-specific antigens such as early secreted antigenic target (ESAT)-6 and culture filtrate protein (CFP)-10 to detect memory T cells that after activation release the cytokine IFN-□. The downside of IGRAs is the inability to differentiate between LTBI, current and past *Mtb* infections. IFN-□ levels during active TB disease and levels after cure of clinical TB disease were not significantly different (Petruccioli et al., 2017). Although a significant difference between LTBI and active TB was found, responses were overlapping between groups making the use of IGRA to distinguish between active and LTBI unfeasible (Carrère-Kremer et al., 2022; Petruccioli et al., 2017).

Independent of whether antibodies are relevant in protection against TB or not, antibodies could present useful biomarkers in TB diagnosis. Current serological TB diagnostics tools are highly variable with suboptimal sensitivity and specificity (Steingart et al., 2011). For this reason, the WHO issued its first negative recommendation, discouraging the use of current commercial serological tests (Steingart et al., 2011; World Health Organization, 2011). Nevertheless, the WHO encourages further research in humoral immune responses to improve serological tests for TB.

The key to an accurate antibody-based test could depend on the selection of appropriate antigens and isotypes. In Pakistani TB patients, a blood-based test, detecting eleven *Mtb*-specific IgG responses, achieved an overall sensitivity of 91% [95% in sputum-smear negative and 88% in smear-negative individuals respectively] and a specificity of 96% when compared to patients with chronic obstructive pulmonary disease (COPD) and 91% to healthy participants respectively (Khaliq et al., 2017). The accuracy of diagnostics could be improved by combining several isotypes. Whereas LAM-IgG alone only showed a sensitivity of 71.4% and a specificity of 86.6% to distinguish between LTBI and active TB, a combination of LAM-IgG with LAM-IgA improved the accuracy to 86.5% (Baumann et al., 2014). A four-marker signature including, anti-Tpx (Rv1932, a thiol peroxidase) IgG, anti-TB-LTBI IgG (composed of Tpx and L16, a 50S ribosomal protein), anti-MPT64 (a eukaryotic membrane-binding bacterial effector) IgA, and anti-LAM IgA distinguished active TB patients from LTBI participants in South Africa with an accuracy of 100% (Awoniyi et al., 2017).

Several studies demonstrate that antibodies could have the potential to be used in the diagnosis of TB. Utilizing antibodies in a point-of-care test could provide a useful tool to rapidly diagnose active TB cases, allow immediate treatment initiation, and prevent future transmission of *Mtb*.

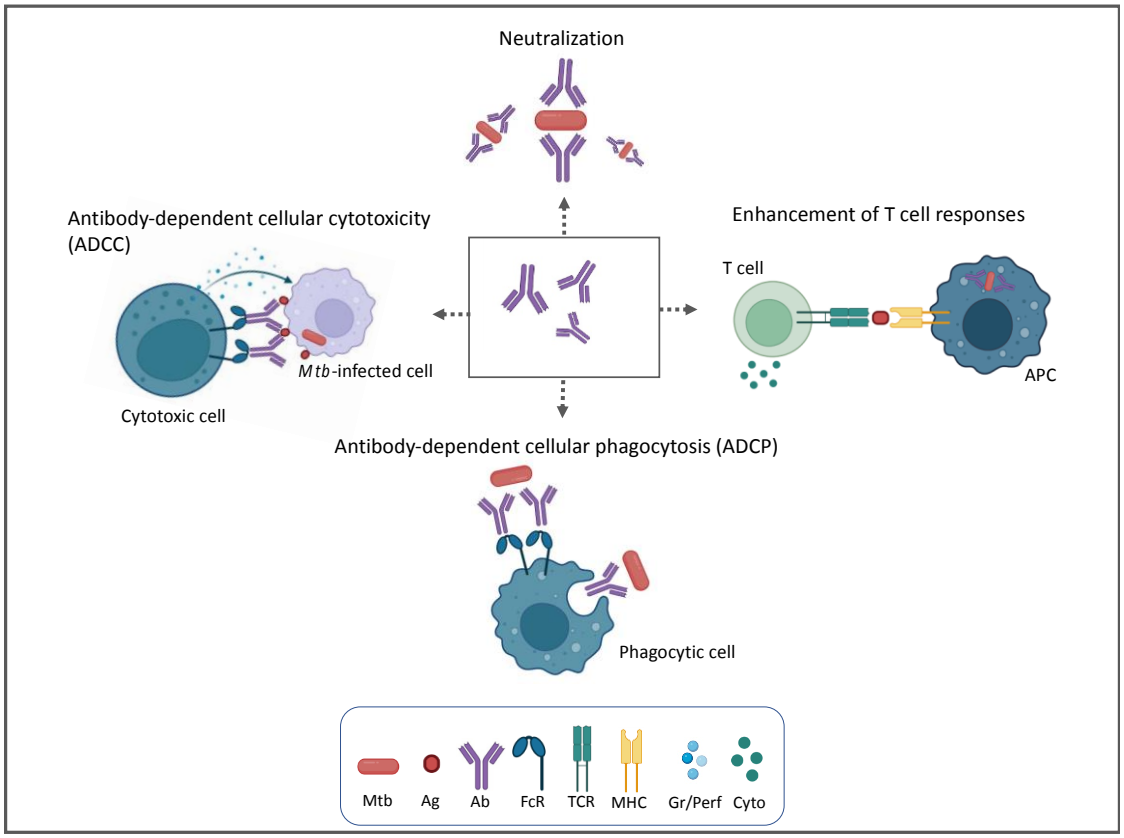

Figure 1: Potential antibody-dependent functions against *Mycobacterium tuberculosis* infection.
Neutralization: *Mycobacterium tuberculosis* (*Mtb*)-specific antibody may bind *Mtb* and prevent uptake into host cells. Enhancement of T cell responses: Immune complexes of antibody (Ab) coated *Mtb* could improve phagocytosis by antigen-presenting cells (APCs), antigen (Ag) processing and presentation via major histocompatibility complex (MHC) to the T cell receptor (TCR) on T cell to enhance effector responses such as cytokine (Cyto) secretion. Antibody-dependent cellular phagocytosis (ADCP): Opsonized *Mtb* bacilli could activate phagocytic cells to kill engulfed *Mtb* bacilli. Antibody-dependent cellular cytotoxicity (ADCC): Antibody could recognize *Mtb*-infected cells and activate cytotoxic cells by crosslinking Fc-receptors (FcR). Cytotoxic cells release granzymes (Gr) and perforin (Perf) to create holes in the membrane of the target cells and kill the infected cell. (Figure created with Biorender.com)

Conclusions

In vitro studies support a functional role of antibodies in protection against *Mtb*, nevertheless, their physiological relevance to protect against TB disease remains to be investigated. Evidence from studies using the current TB vaccine, BCG, show promising evidence that protective antibody responses can be induced by TB vaccine strategies. Targeting antibody responses in new vaccine strategies may be required to enhance overall protective efficacy and support cellular immune responses against TB. Independent of functionality, antibodies may present a useful tool in TB diagnosis. Studies demonstrated promising results in using a combined selection of different isotypes and antigen specificities to develop an accurate diagnostic test for TB. Recent and past studies highlighted the complexity of mycobacteria-specific antibody responses. Further investigation into mycobacteria-specific antibody responses is needed to understand their potential utility in TB vaccines and diagnosis.

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Conflicts of Interest

The authors state no conflict of interest.