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Predictive value of serum bradykinin and desArg^9-bradykinin levels for chemotherapeutic responses in active tuberculosis patients: A retrospective case series

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A B S T R A C T

Background: There is an urgent need for methods that can rapidly and accurately assess therapeutic responses in patients with active tuberculosis (TB) in order to predict treatment outcomes. Exposure to bacterial pathogens can rapidly activate the plasma contact system, triggering the release of bradykinin (BK) and its metabolite desArg^9-bradykinin (DABK) to induce inflammation and innate immune responses. We hypothesized that serum BK and DABK levels might act as sensitive immune response signatures for changes in *Mycobacterium tuberculosis* (*Mtb*) burden, and therefore examined how serum levels of these markers corresponded with anti-TB therapy in a small cohort of active TB cases.

Methods: Nanotrap Mass-Spectrometry (MS) was used to analyze serial blood specimens from 13 HIV-negative adults with microbiologically confirmed active TB who were treated with first-line anti-TB chemotherapy. MS signal for BK (*m*/*z* 1060.5) and DABK (*m*/*z* 904.5) serum peptides were evaluated at multiple time-points (before, during, and after treatment) to evaluate how BK and DABK levels corresponded with disease status.

Results: Serum BK levels declined from pretreatment baseline levels during the early stage anti-TB therapy (induction phase) and tended to remain below baseline levels during extended treatment (consolidation phase) and after therapy completion. BK levels were consistent with induction phase sputum culture conversions indicative of decreased *Mtb* burden reflecting good treatment responses. Serum DABK levels tended to increase during the induction phase and decrease at consolidation and post-therapy time points, which may indicate a shift from active disease to chronic inflammation to a disease free state. Elevated BK and DABK levels after treatment completion in one patient may be related to the subsequent recurrent TB disease.

Conclusions: Our pilot data suggests that changes in the circulating BK and DABK levels in adult TB patients can be used as potential surrogate markers of the host response both early and late in anti-TB treatment for both pulmonary and extrapulmonary TB patients. We will further exploit these host-response signatures in the future as biomarkers in combination with other clinical and microbiologic tools which may improve treatment efficacy and facilitate the development of host-directed therapy.

1. Introduction

Effective treatment of active tuberculosis (TB) requires a multi-antibiotic drug regimen that is able to eliminate *Mycobacterium tuberculosis* (*Mtb*) bacilli and persistent subpopulations of

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intermittently metabolizing bacilli [1]. Although current chemotherapeutic regimens are known to achieve relapse-free outcomes in a majority of patients with drug-susceptible *Mtb*, about 5% of TB patients will have recurrent TB within 2 years of treatment completion [2,3]. Multidrug-resistant (MDR) TB is estimated to account for 3.3% of incident cases and 20% of all cases [2], indicating that MDR TB represents a growing public health threat. Faithful adherence to a treatment regimen with an effective combination of drugs, dosages and treatment duration is one of the most efficient ways to prevent MDR TB development [4]. However, recent bacteriologic and pathology evidence highlights the existence of highly heterogeneous pulmonary *Mtb* lesions (PTB), where lesion growth, structure, and microenvironment may differentially affect antibiotic penetration and access [5]. This phenomenon may partially explain why no single antibiotic is able to eliminate all drug-susceptible mycobacterial subpopulations and why some patients develop recurrent TB diseases after long-term treatment. Multiple high-dose antibiotics are commonly used to ensure patient cures, which can lead to frequent side-effects that contribute to poor treatment adherence and thereby promote treatment failure and/or MDR TB development.

Sputum culture conversion (SCC) after 2 months of treatment is used to analyze initial treatment efficacy in culture positive PTB patients during the induction phase, but sputum culture is not quantitative and cannot assess the extent of *Mtb* bacilli remaining during the consolidation stage when sputum cultures are negative [6]. Most other TB diagnostic assays are also not appropriate for treatment evaluation or require optimization and additional validation before they can be used for this purpose. Xpert MTB/RIF does not differentiate between live and dead bacilli, yielding high false positive results, and thus is not recommended to replace standard smear microscopy and culture methods for monitoring treatment response [7]. Persistently positive interferon-gamma release assays results may indicate a failure of therapy, but this approach has not proven useful for monitoring treatment response in large and longitudinal studies, which found assay reversion results in individuals with or without clinical response to treatment [8,9]. There is thus an urgent need for new serum-based methods that can rapidly predict therapeutic responses and the risk of disease recurrence to improve treatment efficacy and reduce the development of new MDR TB cases.

Most *Mtb* assays detect either *Mtb* bacilli (e.g. AFB smear, mycobacterial culture, Xpert MTB/RIF assays) or ex vivo host responses to *Mtb* antigens (e.g. interferon gamma (IFN-γ) release assays) as a means for TB diagnosis; however, blood levels of host response biomarkers may also yield useful diagnostic information. The plasma contact system, also known as the kallikrein/kinin system, plays a role in the initiation of acute inflammation and maintenance of chronic inflammation during certain infectious diseases [10–12]. Interaction of circulating Factor XII with specific microbial pathogens leads to contact activation and causes a cascade of proteolytic activation steps that ultimately results in the cleavage of high-molecular weight kininogen (HK) to liberate the pro-inflammatory peptide bradykinin (BK) and its metabolite desArg^9-BK (DABK), which play important roles in inflammation responses and represent important surveillance and protective functions in defense against bacterial infections (Fig. 1) [13–15]. However, many bacterial proteinases can also trigger BK production, either by activating kallikrein to stimulate HK cleavage or directly cleaving HK to increase BK release [16,17]. Regardless of its origin, BK-mediated signaling through the kinin B2 receptor (B2R), can activate alveolar macrophages, induce neutrophils and monocyte chemotaxis [18,19], stimulate the migration of immature human monocyte-derived dendritic cells (DC) to inflammation sites [20], induce DC maturation and trigger pro-inflammatory CD4$^+$ Th1 responses [21], including the release of IFNγ and IL-2, which have positive effects on DC maturation and cytotoxic T lymphocytes (CTL) function [22]. Interaction of BK with B2R increases vasodilation and vascular permeability [23], a scenario that might be expected to favor *Mtb* invasion and colonization. BK is rapidly inactivated by circulating hydrolases, particularly angiotensin converting enzyme; however, removal of the C-terminal BK arginine residue, by carboxypeptidase N (CPN) in circulation or carboxypeptidase M (CPM) at sites of inflammation, produces DABK, which also has proinflammatory activity but exhibits a much longer half-life (~20-fold) in serum [24,25]. Emerging evidence indicates that BK-mediated signaling through the B2R dominates the acute phase of bacterial infection while DABK signaling through the kinin B1 receptor (B1R) is involved in chronic inflammation [23,26]. B1R expression can be induced by inflammation [27] or chronic bacterial infection [15], which may enhance DABK signaling to promoting an increase in the production of inflammatory mediators, and is upregulated during persistent inflammation in animal models [25,28]. CPM upregulation accounted for an increase in DABK and B1R agonists in bacterial lipopolysaccharide induced vascular inflammation [29], and soluble CPM can be released from plasma membranes by bacterial phosphatidylinositol-specific phospholipase C activity, which is known to contribute to *Mtb* virulence [30]. DABK-induced B1R signaling can induce vascular smooth muscle cells growth [31], leukocyte accumulation [32] and modulate the life span of neutrophils at sites of inflammation [33]. We therefore hypothesized that pro-inflammatory BK and DABK (Fig. 1) levels might differentially correspond with changes in bacterial load during active TB disease and responses to anti-TB therapy.

To address this hypothesis we used an approach that combined nanotrap-based peptide enrichment and matrix-assisted laser desorption/ionization time-of flight-mass spectrometry (MALDI-TOF-MS) analysis [34,35] to rapidly and accurately detect BK and DAPK peptides in small (5 μl) serum samples of TB patients receiving first-line anti-TB chemotherapy. We systematically evaluated patient BK and DABK levels, clinical characteristics, treatment regimens and therapy outcomes. Our results suggest that changes in BK and DABK serum levels in these patients are related to their anti-TB treatment response.

2. Materials and methods

2.1. Patients

Medical records from the Houston Tuberculosis Initiative (HTI) database [36,37] were retrospectively reviewed, and archived serum samples from 13 adult patients with microbiologically-confirmed tuberculosis and without history of an immunosuppressive condition were obtained for analysis of BK and DABK levels. All study subjects were notified of the potential risks of participation and had given written informed consent, were recruited, enrolled, had specimens obtained and analyzed using Institutional Review Board (IRB)-approved questionnaires, consent forms and procedures. This study was approved by the IRB of the Houston Methodist Hospital, USA (Pro00000546, Pro00005327) and Baylor College of Medicine and its Affiliates, Houston, Texas, USA (H-8182). Diagnosis of active TB was defined by the American Thoracic Society/Centers for Disease Control and Prevention (CDC) guidelines [38] based on clinical and bacteriologic evidence. Serum samples were collected from blood draws performed before, during and after anti-TB therapy completion when available. All subjects had pre-treatment samples and at least one on-treatment sample, but several subjects did not have post-treatment blood samples. All patients underwent HIV testing and were reported HIV negative.

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