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Commentary

Identification and validation of host biomarkers for leprosy: A step forward to establish point-of-care tests

Norman Nausch *, Marc Jacobsen

Department of General Pediatrics, Neonatology, and Pediatric Cardiology, University Children's Hospital, Medical Faculty, Heinrich Heine University, Duesseldorf, Germany

For infectious diseases in which direct detection of the pathogen is challenging, the discovery, validation and implementation of host biomarkers can be crucial for fast and reliable diagnosis, to monitor responses of treatment and help to optimize treatment regimes. In addition, biomarkers can be helpful in reducing transmission by early identification of cases and contact screening. These factors are also critical to avoid further spreading of pathogens in mycobacterial diseases including tuberculosis caused by *Mycobacterium tuberculosis* as well as for Buruli ulcer disease caused by *M. ulcerans* and leprosy caused by *M. leprae* both classified as neglected tropical diseases (NTDs).

Tuberculosis is the classical example for a disease, in which a biomarker assay has been successfully implemented. Interferon-gamma (IFN-γ) release assays (IGRAs) are a standard diagnostic tool in high-income countries with low incidence rates. However, limited sensitivity of IGRAs in young children and immune-compromised individuals as well as potentially in some high-endemic countries highlight the need for additional assays and/or more complex biomarker signatures [1,2].

For many diseases, candidates for promising biomarkers have been identified, but validation and implementation remains a major obstacle [3]. This is especially a problem in NTDs (such as Buruli ulcer disease and leprosy) which are common in low-income countries [4]. Here large-scale approaches to verify biomarkers face huge logistic and financial restrictions and implementation is only achievable if biomarkers are applicable for rapid point-of-care (POC) testing. Furthermore, some NTDs are characterised by a low incidence requiring multi-national long-term approaches for validation. This is the case for Buruli ulcer disease and leprosy where the search for biomarkers is ongoing [5,6].

Leprosy is a highly contagious chronic disease mainly affecting the skin and peripheral nerves. Whereas leprosy has been largely eliminated globally, it remains a serious public health problem in few endemic countries with around 200,000 cases annually [8]. The course of the disease is largely determined by host immune responses involving immune polarisation. A mixed T helper type-1 (T_H1) /T_H17 response is associated with bacterial control and characteristic for tuberculoid leprosy [7]. In contrast, an immune-regulatory/T_H2 response (typically induced in helminth infections), inducing an antibody-mediated immune response, is associated with uncontrolled bacteria (multibacillary) characteristic for Lepromatous leprosy [7]. There are several potential biomarkers for the detection of leprosy described with IgM-antibodies against *M. leprae* phenolic glycolipid I (anti-PGL-I IgM) being the most promising [6]. In combination with CCL4, IL-10, IP-10 and CRP may help to cover different disease outcomes [9]. However, the performance and validation of these biomarkers is, particularly for paucibacillary disease, still unsatisfactory hindering implementation.

In a recent article in *EBioMedicine*, van Hooij and colleagues present a study using a comprehensive approach to identify new biomarkers, validate candidates and test applicability for POC testing [10]. This study uses a funnel approach including a discovery cohort and two validation cohorts. In the discovery cohort, supernatants of whole blood cultures in the presence of antigens (comparable to IGRAs) were screened using a multiplex bead array testing for 60 proteins focusing on cytokines, chemokines and growth factors. Six proteins were selected potentially identifying both the paucibacillary and multibacillary form of the disease and applied in a validation cohort. That comprises previously identified biomarkers such as CCL4, IL-10 and an IP-10 as well as the new marker IL-1Rα. In addition, authors selected additional 11 biomarkers with known or assumed diagnostic potential and all together were measured by ELISA. The first validation cohort confirmed eight of the candidates of which seven were detected in 24 h culture even without specific stimulus rendering potential analysis in plasma. The direct use of plasma specimens has the advantage that it omits the requirement of an overnight culture. Hence candidates were tested in plasma samples of validation cohort II in which five were detectable. Those, namely S100A12, CRP, ApoA1, IP-10 and anti-PGL-I IgM, were further tested using a lateral flow assay, which is applicable for POC and even larger field screening approaches. All five markers showed differences comparing multibacillary patients and controls, whereas ApoA1 could additionally identify paucibacillary forms. However, apart from anti-PGL-I IgM, which had sufficient sensitivity and specificity to distinguish multibacillary patients from controls as reported earlier [6], none of the other biomarkers showed satisfactory results in terms of sensitivity and specificity prompting authors to analyse a combined

* Corresponding author.
E-mail addresses: Norman.Nausch@med.uni-duesseldorf.de (Norman. Nausch), Marc.Jacobsen@med.uni-duesseldorf.de (M. Jacobsen).

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five-marker signature. Using this approach, 86% of leprosy patients were identified with comparable results in both paucibacillary and multibacillary patients.

This elegant study by van Hooij and colleagues proves that new biomarkers can be identified using a funnel approach. Of the identified marker, two candidates (ApoA1 and S100A12) were even suitable to use in lateral flow assays and therefore applicable for use in POC testing and for larger screening approaches. Furthermore, the study indicates the power of biomarker signatures and it already provides a certain degree of validation of identified markers. Further studies are required to prove that this specific signature is useful in other endemic areas with a different genetic background of the affected population. In addition, the exposure to other infections including other mycobacteria and/or helminth parasites influencing host immune responses may differ in different areas affecting the outcome. To analyse the value of these markers in identifying potentially infected contacts of leprosy patients and individuals at particular risk of developing disease would be an additional long-term goal.

This study is a step forward heading towards implementations of according POC tests and also nicely shows that even for a NTD with overall low incidence rates, long-term committed projects can bring advances for improved management.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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