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The next generation of molecular tests will challenge existing TB infrastructure in high burden countries http://ow.ly/lNGVB

Claudia M. Denkinger[1,2], Ioana Nicolau[2], Andrew Ramsay[3], Pamela Chedore[4] and Madhukar Pai[2,4]
[1]Division of Infectious Disease, Beth Israel Deaconess Medical Center, Boston, MA, USA. [2]McGill International TB Centre and Dept of Epidemiology, Biostatistics, and Occupational Health, McGill University, Montreal, QC, and [4]Respiratory Epidemiology and Clinical Research Unit, Montreal Chest Institute, Montreal, Montreal, QC, Canada.[3]Bute School of Medicine, St Andrews University, Fife, UK.

Correspondence: M. Pai, McGill University, Dept of Epidemiology and Biostatistics, 1020 Pine Ave West, Montreal, QC H3A 1A2, Canada. E-mail: madhukar.pai@mcgill.ca

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Serum lipids as biomarkers for therapeutic monitoring of latent tuberculosis infection

To the Editor:

The World Health Organization has estimated that more than two billion persons in the world carry latent tuberculosis infection (LTBI). The diagnosis and treatment of LTBI could have an important impact in preventing the development of active tuberculosis (TB), a disease that causes 1.4 million deaths annually [1]. The current standard treatment of LTBI is nine months of isoniazid (INH) [2]. To improve adherence, a shorter regimen of 4 months rifampicin (RIF) is being evaluated in a multicentre randomised trial (CIHR MCT-94831, registered at www.controlled-trials.com/ISRCTN05675547). This ongoing trial offers an opportunity to study potential biomarkers as surrogates of successful prevention of active disease [3].

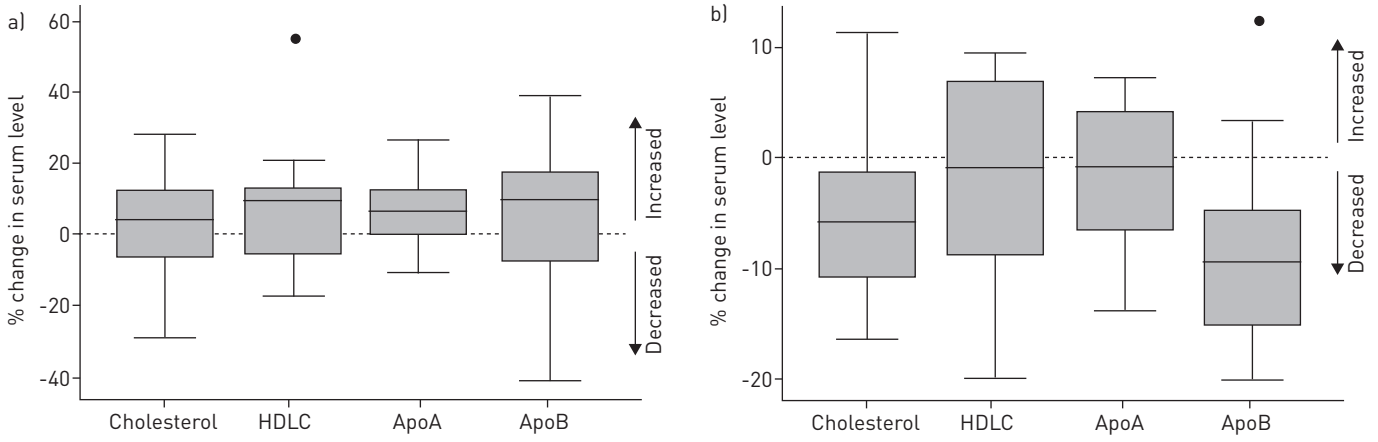

FIGURE 1 Change in serum lipids after 1-month treatment with a) rifampicin and b) isoniazid. HDLC: high density lipoprotein cholesterol; apoA: apolipoprotein A-1; apoB: apolipoprotein B.

Several changes in lipid metabolism could potentially be utilised as biomarkers of effectiveness of LTBI treatment with RIF or INH. RIF is an important stimulator of the pregnane X receptor (PXR), which has been hypothesised to increase the blood levels of high density lipoprotein cholesterol (HDLC) and its protein component apolipoprotein A-1 (apoA) [4, 5]. This hypothesis was supported by observational studies that demonstrated an increased plasma level of HDLC among epileptic patients who were taking anticonvulsant medications which activate the PXR [6]. By contrast, treatment with INH was associated with lower cholesterol blood levels in one small study [7]. ApoA and apolipoprotein B (apoB), a protein component of low density lipoprotein cholesterol (LDLC), have stable serum blood levels without post-prandial changes and low within-individual variability [8]. Total cholesterol and HDLC are inexpensive to measure, reliably measured using non-fasting blood samples, and have lower within-individual variability than triglycerides or LDLC [9].

The objective of this study was to perform a preliminary assessment of total cholesterol, HDLC, apoA and apoB as potential biomarkers, by comparing levels of these four substances before treatment and after one month of treatment with RIF or INH.

From participants in the ongoing multicentre trial of LTBI treatment (CIHR MCT-94831), 15 randomised to 4 months RIF were selected randomly, and 15 randomised to 9 months INH, matched on age (within 2 years) and sex, were selected. LTBI was defined as a positive tuberculin skin test and/or a positive interferon-γ release assay (positive as defined in Canadian TB standards [10]). To limit the variability of metabolic conditions between subjects, we restricted participants to adults between the age of 18 and 45 years. Subjects were excluded if they were consuming alcohol daily or taking other drugs that could induce cytochrome P450 (carbamazepine, phenytoin, phenobarbital, felbamate, topiramate, lamotrigine, griseofulvin, nevirapine and oral contraceptives), as these may influence the effect of RIF on lipid blood levels.

After signing informed consent, participants provided serum samples before treatment and after 1 month of self-administered LTBI treatment. Subjects were not fasting at the time of blood sampling since normal food consumption has no effect on apoA and apoB and a clinically unimportant effect on total cholesterol and HDLC [8]. Serum samples were labelled with study identity numbers and stored at -80°C until assays of total cholesterol, HDLC, apoA and apoB were performed. The identity of participants and study drug taken was known only to one investigator (C. Valiquette); all other investigators remained blinded to study drug for data analysis and interpretation. This study was approved by a research ethics board of the McGill University Health Centre (file number 11-046-SDR).

Treatment effect on lipids was assessed by comparing the post-treatment serum lipids to the pretreatment levels in each arm, using a paired t-test. The mean change in lipid (treatment effect) was compared between the two intervention groups using linear regression statistics. Residual confounding by age and sex, and potential confounding by other variables (body mass index, alcohol consumption and pretreatment lipid levels) were assessed by comparing different regression models, using stepwise backward regression to select the most parsimonious models. A sample size of 30 subjects was considered adequate for detecting a 10–20% change in serum lipids, considering biological variability of 5–20% [9]. Statistical analyses were conducted using STATA v12.1 (StataCorp LP, College Station, TX, USA).

Pretreatment characteristics of the selected subjects receiving INH or RIF were similar. Except for two subjects, all participants had taken more than 80% of their treatment doses during the first month.

INH treatment was associated with a significant decrease in both total cholesterol and apoB levels (mean changes -0.25 $g \cdot L^{-1}$ (95% CI -0.45– -0.06) and -0.07 $g \cdot L^{-1}$ (95% CI -0.12– -0.03), respectively) (fig. 1). The change in serum level of apoB, but not total cholesterol, was significantly different between the two treatment groups, mean difference -0.1 $g \cdot L^{-1}$ (95% CI -0.2– -0.004).

RIF treatment, by contrast, was associated with an increase in mean apoA levels (fig. 1), although this change was not statistically significant (mean change 0.06 $g \cdot L^{-1}$ (95% CI -0.01–0.13)).

These preliminary observations suggest that lipid metabolism may be altered significantly by LTBI treatment, particularly within one month of starting INH. These observations require confirmation in a larger study, but offer the promise of novel biomarkers in LTBI treatment.

This study had several important limitations. First the sample size was small; this limited our ability to identify associations between total cholesterol and INH treatment, or between apoA and RIF treatment. We were unable to evaluate potential drug effects on serum triglycerides and LDLC as we had to rely on non-fasting serum samples. However, measurement of lipids that require 12 h of fasting before drawing samples are not very practical for therapeutic monitoring in clinical practice. In addition, we compared the effect of two different modalities of treatment, but did not include an untreated control group with LTBI to assess the spontaneous changes in these lipid levels, although the finding that RIF did not have a significant effect on apoB serum level suggests that INH was responsible for the changes seen. Finally, we could not exclude confounding by unknown factors. However, since the initial assignment to treatment arms in this patient population was random, confounding by unknown factors should have been unlikely.

Due to lack of clinical symptoms or methods of mycobacterial isolation among patients with LTBI, there is currently no way to evaluate patient response to LTBI treatment. Our finding of a significant association between apoB and INH treatment suggests that changes in serum apoB may be a surrogate of adherence to, and possibly also effectiveness of, INH therapy. However, this requires further evaluation with a larger number of subjects to correlate these changes with patient adherence and effectiveness of INH. In addition, this association, which, to our knowledge, has not been investigated before, may explore a novel mechanism for controlling lipid disorders. Although our observed effect of INH treatment, in reducing apoB level by 8.4%, is less than the effect of statins (at least 20%) and other lipid lowering agents (at least 10%), this effect was observed in healthy individuals with normal baseline apoB levels who are different from those treated with lipid lowering agents.

We conclude that apoB may be a potentially useful biomarker for therapeutic monitoring of LTBI treatment with INH; however, further studies with a larger number of patients, treated for longer periods of time and compared with untreated controls, are required.

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Initial findings that isoniazid treatment reduces apolipoprotein B levels suggest new ways to monitor LTBI therapy http://ow.ly/kR64u

Amr S. Albanna[1], Kenneth Bachmann[2], Donald White[3], Chantal Valiquette[1] and Dick Menzies[1]
[1]Respiratory Epidemiology and Clinical Research Unit, Montreal Chest Institute, McGill University, Montreal, QC, Canada. [2]Dept of Pharmacology, College of Pharmacy, University of Toledo (Emeritus), Toledo, OH, and [3]Dept of Mathematics and Statistics and Dept of Pharmacology, University of Toledo, Toledo, OH, USA.

Correspondence: D. Menzies, Montreal Chest Institute, Room K1.24, 3650 St Urbain Street, Montreal, QC, H2X 2P4, Canada. E-mail: dick.menzies@mcgill.ca

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Major histocompatibility complex class II and *BTNL2* associations in sarcoidosis

To the Editor:

Sarcoidosis is a multiple organ immune-mediated disease of unknown aetiology. Identified genetic risk factors within the major histocompatibility complex (MHC), such as *butyrophilin-like* (*BTNL)2*, human leukocyte antigen (*HLA)-DRB1*03* and *HLA-DRB1*15*, and protective factors, such as *HLA-DRB1*01*, are found in many populations [1, 2]. The vast majority of sarcoidosis patients have a favourable prognosis, but approximately 20% develop a chronic, disabling disease [3].

Chronic beryllium disease (CBD) and chronic sarcoidosis share similarities as granulomatous diseases and they are pathologically indistinguishable from each other. CBD has been associated with *HLA-DPB1*02:01*, especially with a glutamic acid residue at position 69 (Glu69) [4]. A functional splice-site polymorphism rs2076530 within the *BTNL2* gene has been suggested to predispose to sarcoidosis [5]. However, previous studies have shown conflicting results as to whether *HLA-DPB1* also predisposes to sarcoidosis, and whether the *BTNL2* association is a result of linkage disequilibrium with *HLA-DRB1* [6, 7].

The main objective of this study was to evaluate the *HLA-DPB1* polymorphisms and the *BTNL2* splice-site variant in Finnish patients suffering from sarcoidosis followed-up for 5–15 years and clinically categorised into subgroups based on disease prognosis. In addition, we constructed haplotypes containing MHC class II genes (*HLA-DRB1* and *-DPB1*) and rs2076530, and studied the influence of MHC markers and their combinations on disease susceptibility.

We examined a total of 188 patients with verified pulmonary sarcoidosis. The patients were divided into those with a disease resolved within 2 years (n=90) and those with persisting activity at that time point (n=98). The control population consisted of 150 healthy subjects representing the Finnish population. The characteristics of all have been previously reported [8]. All patients and controls gave their written informed consent to participate in the study.

Subjects were typed for *HLA-DPB1* (Invitrogen, Life Technologies, Carlsbad, CA, USA or Olerup SSP AB, Stockholm, Sweden) and rs2076530 (Sequenom, San Diego, CA, USA). In haplotype and linkage disequilibrium analysis the previously published *HLA-DRB1* alleles of the subjects were utilised [8].

Molecular analyses of MHC genes were performed using published protocols [8]. All comparisons were made between four different dichotomous outcome variables: all sarcoidosis patients *versus* controls; patients with resolved disease *versus* controls; patients with persistent disease *versus* controls; and disease prognosis was studied by comparing patients with resolved disease *versus* patients with persistent disease.

Table 1 provides a summary of the analyses. *HLA-DPB1* and rs2076530 loci were in Hardy–Weinberg equilibrium in both cases (*HLA-DPB1*: p=0.17; *BTNL2* rs2076530: p=0.74) and controls (*HLA-DPB1*: p=0.24; *BTNL2* rs2076530: p=0.86) measured directly by the exact test using the Markov-chain approach.