SUMMARY

This thesis, titled ‘A metabolomics investigation of Tuberculous Meningitis in infants and children’, deals with tuberculous meningitis (TBM), the most severe complication of tuberculosis (TB) and major pandemic of our day. The WHO Global TB Report listed approximately 312 380 new cases of TB in 2013 for South Africa alone, of which up to 10% manifested in the central nervous system — particularly severe as TBM in children. Existing TB tests and diagnostic markers have a low sensitivity and specificity, indicating a lack of valid and specific biomarkers for TBM.

We present here the first comprehensive metabolomics investigation using a homogeneous and well-described TBM infant and children patient group, in a thesis structured into four parts.

Part 1 gives the background on clinical aspects of TBM and a biochemical overview with emphasis on host–pathogen interaction, raising a key biological question: “Can biologically relevant metabolic perturbations be identified in the cerebrospinal fluid (CSF) of infants and children with TBM and are these perturbations reflected in the urine through putative biomarkers?” (Chapter 1). The experimental approach to address this question was untargeted proton nuclear magnetic resonance (¹H NMR) spectroscopy and semi-targeted gas chromatography–mass spectrometry (GC-MS) metabolomics (Chapter 2).

Part 2 covers the ¹H NMR component of the investigation, shown to be a highly repeatable method and useful for an initial, holistic assessment of TBM (Chapter 3). CSF was the biofluid studied, as it is derived from close to the site of TBM infection. The new insight(s) gained from the global CSF metabolite profile (first aim of the study), was expressed as the astrocyte-microglia lactate shuttle (AMLS) hypothesis (Chapter 4). This conceptual AMLS model is further discussed and directives given for hypothesis verification. It is noted that ¹H NMR based metabolomics studies offer distinct insight(s) into TB and meningitis (Chapter 5).

Part 3 focuses on the GC-MS related aspects of the thesis. Following a brief review (Chapter 6), a new method is described for the qualitative assessment of the precision by which analysts generate a GC-MS metabolomics data matrix — designated as KEMREP (Chapter 7). GC-MS analysis of urine samples from patients and controls revealed a global metabolite profile that characterized TBM (second aim; Chapter 8). The key distinguishing metabolites for TBM were methylcitric, 2-ketoglutaric, quinolinic and 4-hydroxyhippuric acids — SUM-4 — proposed to be a putative diagnostic TBM biosignature (third aim; Chapter 8).

Part 4 discusses the achievements of the thesis in context of the relevant biological and clinical aspects pertaining to TBM (Chapter 9) — addressing the aims. The investigation concludes (Chapter 10) with perspectives on the limitations and future prospects; illustrated using a targeted ultra-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (UPLC-ESI-MS/MS) method for the determination of the ratio of the L and D enantiomers of lactic acid in CSF samples from TBM patients. This final, follow-up study confirmed that lactic acid in
the CSF of TBM cases was only in the L-form, solely a response from the host to the infection, and provided experimental support to the conceptual AMLS model.

**Keywords:** tuberculous meningitis (TBM); metabolomics; urine; cerebrospinal fluid (CSF); proton nuclear magnetic resonance (1H NMR) spectroscopy; gas chromatography–mass spectrometry (GC-MS); hypothesis; astrocyte-microglia lactate shuttle (AMLS); KEMREP; biosignature; L-lactic acid.

**Format:** This thesis is presented in article format and meets the requirements set out by North-West University, South Africa and Vrije Universiteit, Amsterdam. Thus the following full, peer reviewed papers forms part of the thesis:


