Worldwide, tuberculosis (TB) remains a major health problem with efforts to control TB still hindered by gaps in knowledge that exist in diagnosis, prevention and treatment. After exposure, human infection with *Mycobacterium (M.) tuberculosis* can progress to active disease, be contained as latent infection, or be eradicated by the host immune response. Central nervous system involvement occurs in ~1% of all cases of TB, among which cases of tuberculous meningitis (TBM) are the most severe manifestation and frequently occur during early childhood. Although the clinical presentation and histo-pathological mechanisms of TBM are well-defined over the last decades, the cellular and molecular mechanisms are still poorly understood. A successful host response to an invading pathogen requires precise co-ordination of the components of the immune system. The studies described in this thesis aim to better understand the host immune response to TBM in order to improve future diagnostic, preventive and therapeutic strategies.

**PART I** – In the first part of this thesis the host immune response to TBM and the role of vitamin D is investigated. In Chapter 2 biomarker patterns in cerebrospinal fluid (CSF) and serum of children resident in a TB endemic area, with signs and symptoms suggestive of meningitis, are evaluated by using multiple statistical analyses. Unsupervised hierarchical clustering analysis revealed a disease-specific pattern of biomarkers for TBM relative to other forms of meningitis. Pathway analysis indicated that biomarkers involved in TBM pathogenesis resembled those involved in multiple sclerosis, and that involved in vitamin D receptor / retinoid X receptor activation were over-represented in TBM compared to other forms of meningitis. With these results, a promising CSF biomarker-based diagnostic prediction model for childhood TBM has been developed, based on three biomarkers: interleukin-13, vascular endothelial growth factor, and cathelicidin LL-37. The results of this study highlight the potential of bio-signatures in the host’s CSF for diagnostic applications and for improving our understanding of the pathogenesis of TBM to discover strategies to prevent immune-pathological sequelae.

The incidence rate of TBM varies with season, and serum vitamin D levels, which are dependent on sunlight, may play a role. Chapter 3 describes the association found between the seasonal variation in the incidence rate of TBM and sunshine hours prior to disease manifestation. This association supports the hypothesis that vitamin D has a role in the pathophysiology of TBM. In Chapter 4 a prospective study is described in children with suspected meningitis in which serum 25-hydroxyvitamin D (25(OH)D) levels and CSF concentrations of cathelicidin LL-37 and five other vitamin-D related biomarkers were investigated. Low serum 25(OH)D levels were
found to be associated with a TBM diagnosis, while CSF cathelicidin LL-37 concentrations are relatively high in TBM patients when compared to non-TBM patients. Furthermore, significant correlations were found between cathelicidin LL-37 and concentrations of interleukin (IL)-13, Interferon-\(\gamma\) (IFN-\(\gamma\)), Regulated on activation normal T cell expressed and secreted (RANTES) and IFN-\(\gamma\)-induced protein-10 in CSF. All together, these findings stress the role of vitamin D in the pathophysiology of TBM.

Currently, the best explanation for low vitamin D levels in patients with active TB is that a fall in serum 25(OH)D concentration activates latent disease. As vitamin D is mainly derived from cutaneous photosynthesis in the presence of Ultraviolet-B radiation, its production declines to a minimum during winter months in countries at higher latitudes. Immigrants to these countries are known to be at increased risk for vitamin D deficiency and consequently for \textit{M. tuberculosis} disease activation. In the last chapter of part one, the association between migration status and vitamin D deficiency in a Dutch paediatric population is studied (Chapter 5). Differences in serum vitamin D levels were found between native Dutch and non-Western immigrants, with lowest vitamin D levels in first-generation non-Western immigrants.

\textbf{PART II} – The second part of this thesis focuses on improving the early diagnosis of childhood TBM and the impact of drug resistance on clinical outcome. The main difficulty with diagnosis of TBM in children is its paucibacillary nature. Among the most promising antigen-detection assays for diagnosing TB is an assay based on the detection of lipoarabinomannan (LAM), a \textit{Mycobacterium}-specific lipopolysaccharide of the bacillus cell wall. Chapter 6 describes the diagnostic accuracy of a commercial urine LAM antigen-detection assay. In contrast with results of studies done in adults, urinary LAM detection appeared to be of little diagnostic value for the diagnosis of TBM in a paediatric population of meningitis suspects (sensitivity 4.8% and specificity 93.1%). Given the inverse relationship between CD4\(^+\) T-cell count and urinary LAM sensitivity, the low number of HIV-infected TBM patients in our study cohort could have contributed to the low sensitivity observed. In the study presented in Chapter 7 we prospectively assessed the diagnostic accuracies of two commercial nucleic acid amplification tests (NAATs, Genotype MTBDR\textit{plus}® and Xpert MTB/RIF®) on CSF collected from paediatric meningitis suspects. Using a pre-defined TBM case-definition as reference standard, sensitivities and specificities were 32% and 98% for MTBDR\textit{plus}®, and 25% and 100% for Xpert MTB/RIF®. The combination of the two NAATs gave the best diagnostic performance with a combined sensitivity of 48% and a specificity of 98%. Given the low sensitivities of CSF microscopy and liquid culture, the improved diagnostic performance achieved with commercial NAATs is encouraging.
During the past 5 years, an alarming increase in the number of patients with multidrug-resistant (MDR)-TB and extensively drug-resistant TB has been noted. In the last chapter of Part II, the impact of drug resistance on clinical outcome in children with TBM is discussed (Chapter 8). MDR-TBM in children seems to have poor clinical outcome and is associated with death. No difference in the outcomes between children with isoniazid mono-resistant TBM and those with drug-susceptible TBM were found. A probable explanation for this finding could be due to the anticipation of isoniazid mono-resistance by the local TBM treatment regimen used in this study.