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Cerebrospinal fluid IL-1β is elevated in tuberculous meningitis patients but not associated with mortality

Valerie A.C.M. Koeken [a,b], Ahmad R. Ganiem [c], Sofiati Dian [c], Rovina Ruslami [c], Lidya Chaidir [c], Mihai G. Netea [a,d], Vinod Kumar [a,e], Bachti Alisjahbana [c], Reinout van Crevel [a,f], Arjan van Laarhoven [a,*]

[a] Department of Internal Medicine and Radboud Center for Infectious Diseases (RCI), Radboud Institute for Molecular Life Sciences (RIMLS), Radboud University Medical Center, Nijmegen, the Netherlands
[b] Department of Computational Biology for Individualised Infection Medicine, Centre for Individualised Infection Medicine (CiiM) & TWINCORE, Joint Ventures Between the Helmholtz-Centre for Infection Research (HZI) and the Hannover Medical School (MHH), 30625 Hannover, Germany
[c] TB-HIV Research Center, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
[d] Department for Genomics & Immunoregulation, Life and Medical Sciences Institute (LIMES), University of Bonn, 53115 Bonn, Germany
[e] Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands
[f] Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK

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ABSTRACT

Inflammation contributes to the pathophysiology and high mortality of tuberculous meningitis. The IL-1β pathway has been implicated in immunopathology and could be a target for host-directed therapy. IL-1β was elevated in the cerebrospinal fluid (CSF) of 225 HIV-uninfected tuberculous meningitis patients in Indonesia compared to controls, but did not predict subsequent mortality, nor did IL-6 or IL-1Ra. Furthermore, genetic loci known to regulate *IL1B* gene expression did not predict mortality in 443 tuberculous meningitis patients, although two of these loci did predict CSF IL-1β concentrations. Collectively, these data argue against a role for IL-1β targeted host-directed therapy in tuberculous meningitis.

1. Introduction

Tuberculous meningitis is the most devastating manifestation of tuberculosis, leading to high rates of morbidity and mortality in those affected. Inflammation plays an important role in the pathophysiology of tuberculous meningitis [1], as adjunctive corticosteroids reduce mortality [2]. The immune response shows clear differences between blood and cerebrospinal fluid (CSF) [3]. Therefore, understanding the cerebral inflammatory response is crucial to improve therapeutic strategies.

The immune system is thought to contribute to poor outcome of tuberculous meningitis, either through inadequate killing of *Mycobacterium tuberculosis*, or through excessive inflammation leading to tissue damage, so called immunopathology [1]. Adjunctive host-directed immune interventions should therefore either enhance protective immunity or regulate pathological tissue-damaging immunity. To study the pathophysiological inflammatory responses in tuberculous meningitis, we can learn from patients with immune reconstitution inflammatory syndrome (IRIS), which can occur after antiretroviral therapy is initiated in tuberculosis-HIV co-infected patients. Whole blood transcriptomic analysis comparing tuberculous meningitis IRIS patients to non-IRIS controls revealed increased activation of inflammasome genes [4]. Inflammasome activation leads to cleavage and secretion of the pro-inflammatory cytokine IL-1β. Even though the study by Marais et al. was conducted in HIV-infected tuberculous meningitis IRIS patients, we hypothesize that targeting the pro-inflammatory IL-1β pathway could also potentially be beneficial in HIV-uninfected tuberculous meningitis patients, for example with interleukin receptor 1 inhibitors anakinra, which targets IL-1α and IL-1β. Anakinra penetrates well into the central nervous system and has been successful in treating central nervous system inflammation [5,6]. In herpes simplex virus encephalitis, IL-1β levels showed a relationship to clinical severity and outcome [7]. In

Abbreviations: CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; IRIS, immune reconstitution inflammatory syndrome; IQR, interquartile range; QTL, quantitative trait loci; SNP, single nucleotide polymorphism.

* Corresponding author. Geert Grooteplein 8, 6525 GA Nijmegen, Netherlands.
E-mail address: Arjan.vanLaarhoven@radboudumc.nl (A. van Laarhoven).

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HIV-uninfected tuberculous meningitis patients, however, definitive data on CSF IL-1β and its relation to disease severity and outcome are lacking [8,9].

We therefore assessed the role of IL-1β in tuberculous meningitis, by linking CSF concentrations of several cytokines of the IL-1 family and genetic regulators of CSF IL-1β to disease severity and mortality in a large cohort of HIV-uninfected patients.

2. Methods

2.1. Study design and participants

All study participants were included in a prospective cohort of patients with subacute meningitis suspected for tuberculous meningitis from the Hasan Sadikin Hospital in Bandung, Indonesia, from October 31, 2006 to June 16, 2016. All patients in this cohort fulfilled the clinical criteria for suspected tuberculous meningitis as previously described [10]. Definite tuberculous meningitis was defined by a positive CSF *M. tuberculosis* culture or PCR. Probable tuberculous meningitis cases had a CSF leukocyte count ≥5 per μL and a CSF:blood glucose-ratio < 0.5, but negative CSF *M. tuberculosis* culture and PCR. Controls underwent a lumbar puncture because of suspected meningitis, but had a negative *M. tuberculosis* culture and PCR, and normal routine CSF characteristics. HIV co-infection, which strongly affects the immune response and mortality of tuberculous meningitis patients, was an exclusion criterion for the study. Routine clinical and CSF characterization were performed at the time of diagnosis, and survival was monitored for six months [11]. Patients were included under the project "Optimization of Diagnosis of Meningitis", approved by the Ethics Committee of Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia (449/UN6.C1.3.3/KEPK/PN/2015).

2.2. Protein analysis

CSF samples were centrifuged for 15 min at 3000 rpm (1400×*g*) and the supernatant was stored at −80 °C. IL-1β was measured in 225 CSF samples using the Simple Plex cartridges run on the Ella platform (ProteinSimple, San Jose, California, USA) following the manufacturer's instructions. IL-1Ra, IL-1α and IL-6 were measured in only 131 samples due to practical limitations. IL-1Ra was measured using enzyme-linked immunosorbent assay (ELISA) and was performed according to the manufacturer's protocol (R&D Systems, Minneapolis, Minnesota, USA). IL-1α and IL-6 were measured using the commercially available ProSeek Multiplex Inflammation I panel (Olink Proteomics, Uppsala, Sweden). The procedure of the multiplex proximity extension assay was performed as previously described [12]. Only proteins with detectable levels in at least 75% of the samples of tuberculous meningitis patients were included in the analysis.

2.3. Genotype analysis

Genotyping and imputation were performed as previously described [13]. Single nucleotide polymorphisms (SNP) with an imputation score (R^2) > 0.8 and minor allele frequency ≥ 0.1 were included in the analysis. SNPs that significantly regulate the expression of *IL1B*, known as expression quantitative trait loci (eQTL), were identified from eQTLGen Consortium (false discovery rate < 0.05), which incorporates 37 whole blood datasets (available at www.eqtlgen.org) [14]. To filter for linkage disequilibrium, SNPs were correlated using Spearman's Rank-Order correlation and clustered using average hierarchical clustering using a R^2 of 0.6 as a cut-off. Each cluster was represented by the SNP with the lowest distance to the other traits in that cluster.

2.4. Statistical analysis

All computational analyses were performed in R 3.3.3. Cytokine concentrations were log-transformed before analysis. The predictive value of cytokines and SNPs on mortality was analyzed using Cox regression with R packages 'survival' and 'survminer'. The association between IL-1β and IL-1β eQTLs was assessed in a linear regression model including age and sex as covariates. A p-value of less than 0.05 was considered statistically significant.

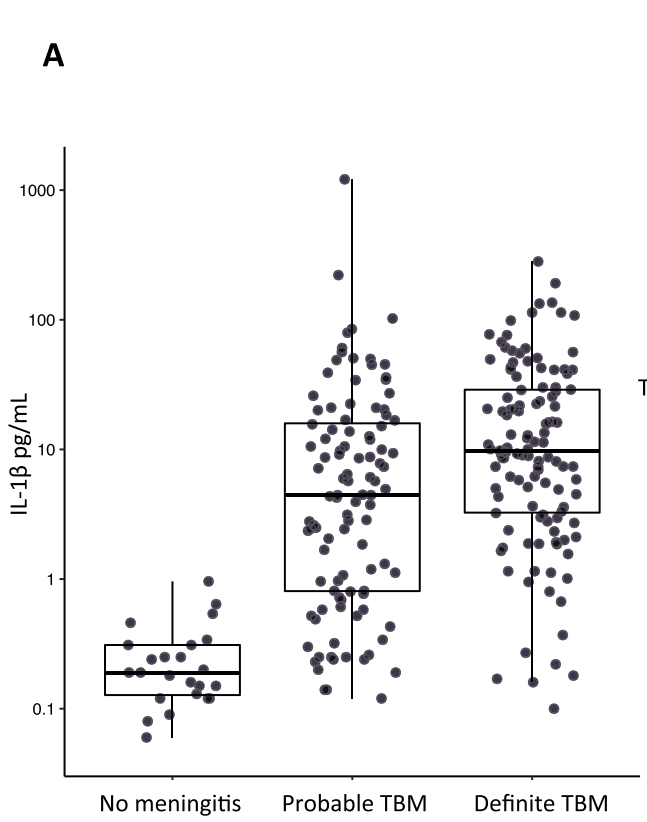
3. Results

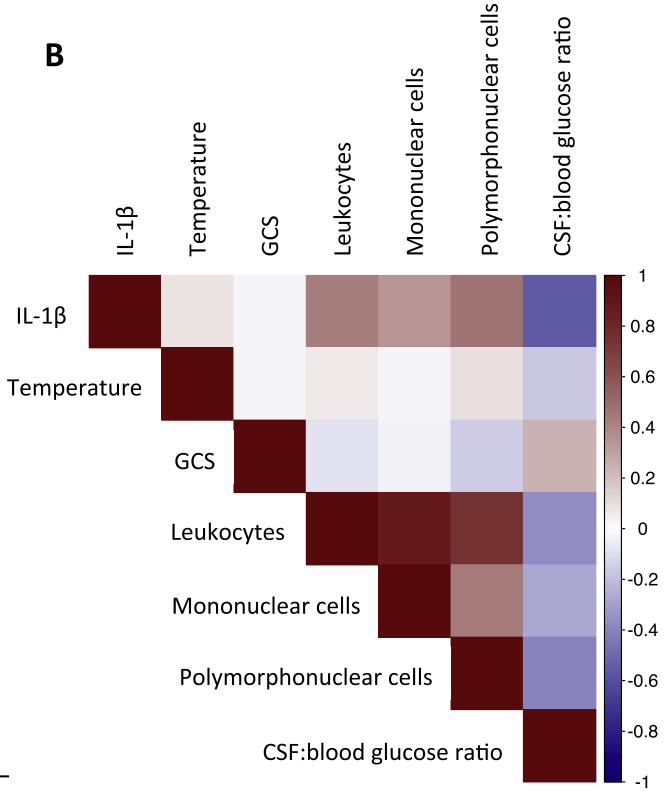
For analysis of IL-1β concentration in the CSF, a total of 225 HIV-uninfected tuberculous meningitis patients and 24 controls were included, while IL-1α, IL-1Ra and IL-6 were measured in 131 tuberculous meningitis patients. Controls had a median age of 32 (interquartile range [IQR] = 22–41), and 54% were male. Patients had a median age of 30 (IQR = 21–38), and 56% were male. Median Glasgow Coma Scale and body temperature were 13 (IQR = 12–15) and 37.6 °C (IQR = 36.8–38.2), respectively. CSF leukocytes count totaled 187 cells/μL (IQR = 70–370), with 110 mononuclear cells/μL (IQR = 40–202) and 37 polymorphonuclear cells/μL (IQR = 10–117). Of the tuberculous meningitis patients, 54% had definite tuberculous meningitis as confirmed by CSF culture or PCR and most presented with severe disease of BMRC grade II (80%) or III (12%). Among 225 tuberculous meningitis patients followed until six months, mortality was 34%. IL-1β was detected in 205 of 225 patients; IL-1Ra and IL-6 were measured in 131 patients and both were detected in at least 85% of the tuberculous meningitis CSF samples. IL-1α had detectable levels in only 44% of the samples, and was therefore excluded from the analysis.

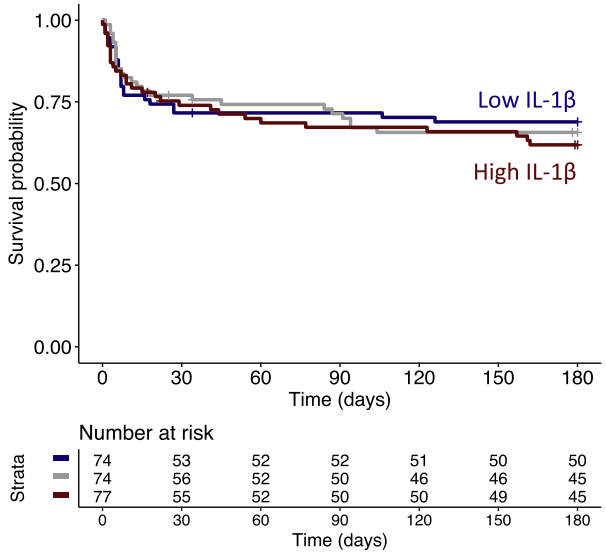
IL-1β concentrations in the CSF were 44-fold higher in tuberculous meningitis cases compared to controls (median 8.34 pg/mL vs. 0.19 pg/mL), and significantly higher in probable ($p < 0.0001$) as well as definite tuberculous meningitis cases ($p < 0.0001$, Fig. 1A). IL-1β levels in tuberculous meningitis patients did not correlate with Glasgow Coma Scale nor with body temperature, but showed a positive correlation with total CSF leukocyte (Spearman's $\rho = 0.44$), mononuclear ($\rho = 0.34$) and polymorphonuclear cell counts ($\rho = 0.48$), and a negative correlation with CSF to blood glucose ratio ($\rho = -0.55$, all mentioned Spearman correlations $p < 0.0001$, Fig. 1B).

Although elevated, CSF IL-1β concentrations did not predict 30-day or 180-day mortality (Fig. 1C). To further look into the IL-1 pathway, we also analyzed the receptor antagonist IL-1Ra, and IL-6, which is a downstream target of IL-1. CSF IL-1Ra concentrations were undetectable in most controls, and were at least 26-fold higher in tuberculous meningitis patients compared to controls ($p < 0.0001$), and strongly correlated with CSF IL-1β. However, IL-1Ra levels did not predict survival, nor did the IL-1β/IL-1Ra ratio, as a measure of bio-active IL-1β. CSF IL-6, as a downstream target of IL-1β, was 936-fold higher in tuberculous meningitis patients compared to controls ($p < 0.0001$), but also did not predict mortality during follow-up (Fig. 1D).

Finally, to further investigate the association between IL-1β levels and outcome in tuberculous meningitis, we examined if SNPs that regulate *IL1B* gene expression, also called expression quantitative trait loci (eQTL), correlate with CSF IL-1β and patient mortality. From a meta-analysis of whole blood eQTLs [14], we identified 102 eQTLs within 1 Mb distance of the *IL1B* gene (*cis*-eQTLs), of which 81 were present in our genome-wide SNP typing data. Similarly, we selected 41 eQTLs that regulate *IL1B* expression but are located distant from the *IL1B* gene (*trans*-eQTLs), of which 33 SNPs were present in our dataset. Removal of SNPs that were in linkage disequilibrium with each other resulted in 6 representative *cis*-eQTLs and 11 representative *trans*--eQTLs. Of those 6 *cis*-eQTLs, 2 were associated with CSF IL-1β concentrations, but none were associated with 180-day mortality (N = 443 with 171 events; Table 1). In addition, none of the 11 *trans*-eQTLs predicted CSF IL-1β levels in tuberculous meningitis patients.







D

	Correlation with IL-1β		Association with survival	
	Rho	**p**	**HR (95% CI)**	**p**
IL-1Ra	0.79	< 0.0001	0.96 (0.82-1.12)	0.58
IL-6	0.76	< 0.0001	0.98 (0.89-1.07)	0.66

Fig. 1. (**A**) Cerebrospinal fluid (CSF) IL-1β concentrations of controls without meningitis (N = 24), and patients with probable (N = 103) and definite (N = 122) tuberculous meningitis (TBM). (**B**) Correlation matrix based on Spearman correlations on pairwise complete observations in tuberculous meningitis patients. IL-1β concentrations and leukocyte, mononuclear and polymorphonuclear cell counts were measured in the CSF. (**C**) Kaplan-Meier graph with 180-day survival for patients (N = 225) based on CSF IL-1β concentrations divided in tertiles. The groups were defined by the following cut-offs: low (IL-1β < 3.78 pg/mL, depicted in blue), intermediate (IL-1β 3.78–13.9 pg/mL, depicted in grey) and high (IL-1β > 13.9 pg/mL, depicted in red). (**D**) Table summarizing the Spearman correlations between IL-1β, and IL-1Ra and IL-6 concentrations in the CSF (N = 101), and the results of the univariate cox regression models for predicting six-month mortality (N = 130). The hazard ratio (HR) and 95% confidence interval (CI) are shown. Abbreviations: CI, confidence interval; CSF, cerebrospinal fluid; GCS, Glasgow Coma Scale; HR, hazard ratio; TBM, tuberculous meningitis. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
IL1B eQTLs and their link with CSF IL-1β levels in tuberculous meningitis patients.

eQTL	Chromosome	Minor allele	Major allele	MAF	Type	Association with CSF IL-1β		
						beta	*p-value*	*FDR*
rs4848287	2	T	C	0.31	*cis*-eQTL	0.02	0.815	0.815
rs4337479	2	A	G	0.19	*cis*-eQTL	0.07	0.423	0.521
rs2222131	2	T	G	0.19	*cis*-eQTL	0.20	0.033	0.099
rs6542079	2	C	T	0.18	*cis*-eQTL	0.21	0.025	0.099
rs13027814	2	A	G	0.34	*cis*-eQTL	0.06	0.434	0.521
rs45550936	2	G	A	0.10	*cis*-eQTL	0.14	0.203	0.406

Significant eQTLs for *IL1B* were identified from eQTLGen Consortium (available at www.eqtlgen.org) [14]. Abbreviations: FDR, false discovery rate; eQTL, expression quantitative trait loci; MAF, minor allele frequency.

4. Discussion

Tuberculous meningitis remains the most severe manifestation of tuberculosis, with high morbidity and mortality rates. In cases where adjunctive corticosteroids are ineffective, alternative host-directed interventions could potentially improve outcome of tuberculous meningitis patients. IL-1β has been implicated in immunopathology in tuberculous meningitis and could be a promising target for host-directed therapy. Its drug antagonist, anakinra, shows good central nervous system penetration [5]. Therefore, we investigated the association between IL-1β levels and mortality in tuberculous meningitis. However, although CSF IL-1β concentrations were elevated among 225 HIV-uninfected tuberculous meningitis patients in Indonesia, its levels did not predict mortality. As further evidence against a possible role for IL-1β, genetic loci known to regulate *IL1B* expression did not predict mortality in 443 tuberculous meningitis patients, although two of these loci did predict CSF IL-1β concentrations.

A whole blood transcriptomic analysis studying the pathophysiological inflammatory responses in HIV-infected tuberculous meningitis IRIS revealed a central role for the activation of inflammasome genes [4]. This highlights the importance of innate immune responses in damaging inflammation in tuberculous meningitis, since inflammasome activation plays a central role in innate immunity and leads to cleavage and secretion of IL-1β. Although IL-1β levels were elevated in the CSF of tuberculous meningitis patients in our study, we did not observe an association between IL-1β levels and fever or Glasgow Coma Scale score, and these levels did not predict mortality. These results do not support routine use of drugs targeting IL-1β in HIV-uninfected tuberculous meningitis patients. This does not exclude the possibility of a beneficial effect in selected patients, such as those with vasculitis or a protracted paradoxical reaction [15]. Moreover, our results cannot be extrapolated to HIV-infected patients or tuberculous meningitis IRIS patients. All of this will require further study.

Our study has several strengths, including its size (N = 225 for cytokines, N = 443 for genetics), high rate of microbiologically confirmation (54%), and prospective follow-up of patients. Limitations of our study include that we could not assess levels of IL-1β and other cytokines in the brain parenchyma and the systemic circulation. In addition, our analysis was limited to baseline samples and we did not study kinetics after start of treatment. Lastly, this is an observational study, based on which we cannot exclude that anti-IL-1 treatment with anakinra improves tuberculous meningitis outcome. However, randomized trials are costly and time-consuming, which underlines the importance of observational studies to prioritize potential therapeutic targets.

In conclusion, IL-1β levels in the CSF are elevated but not related to mortality in HIV-uninfected tuberculous meningitis patients, and targeting the IL-1 pathway is therefore unlikely to improve patient outcome. An unbiased approach, such as we have applied successfully using CSF metabolomics revealing an essential role for tryptophan metabolism [16], may be needed to identify inflammatory pathways as potential targets for adjunctive host-directed therapy.

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5. Potential conflicts of interest

The authors declare that there is no conflict of interest.

CRediT authorship contribution statement

Valerie A.C.M. Koeken: Conceptualization, Data curation, Methodology, Formal analysis, Writing - original draft. **Ahmad R. Ganiem:** Investigation, Writing - review & editing. **Sofiati Dian:** Investigation, Data curation, Formal analysis, Writing - review & editing. **Rovina Ruslami:** Supervision, Writing - review & editing. **Lidya Chaidir:** Investigation, Writing - review & editing. **Mihai G. Netea:** Conceptualization, Supervision, Funding acquisition, Writing - review & editing. **Vinod Kumar:** Formal analysis, Supervision, Methodology, Writing - review & editing. **Bachti Alisjahbana:** Conceptualization, Supervision, Funding acquisition, Writing - review & editing. **Reinout van Crevel:** Conceptualization, Supervision, Methodology, Funding acquisition, Writing - review & editing. **Arjan van Laarhoven:** Conceptualization, Supervision, Methodology, Funding acquisition, Writing - review & editing.

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