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Cerebral tryptophan metabolism and outcome of tuberculous meningitis: an observational cohort study

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See Comment page 479

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Summary

Background Immunopathology contributes to the high mortality of tuberculous meningitis, but the biological pathways involved are mostly unknown. We aimed to compare cerebrospinal fluid (CSF) and serum metabolomes of patients with tuberculous meningitis with that of controls without tuberculous meningitis, and assess the link between metabolite concentrations and mortality.

Methods In this observational cohort study at the Hasan Sadikin Hospital (Bandung, Indonesia) we measured 425 metabolites using liquid chromatography-mass spectrometry in CSF and serum from 33 HIV-negative Indonesian patients with confirmed or probable tuberculous meningitis and 22 control participants with complete clinical data between March 12, 2009, and Oct 27, 2013. Associations of metabolite concentrations with survival were validated in a second cohort of 101 patients from the same centre. Genome-wide single nucleotide polymorphism typing was used to identify tryptophan quantitative trait loci, which were used for survival analysis in a third cohort of 285 patients.

Findings Concentrations of 250 (70%) of 351 metabolites detected in CSF were higher in patients with tuberculous meningitis than in controls, especially in those who died during follow-up. Only five (1%) of the 390 metobolites detected in serum differed between patients with tuberculous meningitis and controls. CSF tryptophan concentrations showed a pattern different from most other CSF metabolites; concentrations were lower in patients who survived compared with patients who died (9-times) and to controls (31-times). The association of low CSF tryptophan with patient survival was confirmed in the validation cohort (hazard ratio 0·73; 95% CI 0·64–0·83; p<0·0001; per each halving). 11 genetic loci predictive for CSF tryptophan concentrations in tuberculous meningitis were identified (p<0·00001). These quantitative trait loci predicted survival in a third cohort of 285 HIV-negative patients in a prognostic index including age and sex, also after correction for possible confounders (p=0·0083).

Interpretation Cerebral tryptophan metabolism, which is known to affect *Mycobacterium tuberculosis* growth and CNS inflammation, is important for the outcome of tuberculous meningitis. CSF tryptophan concentrations in tuberculous meningitis are under strong genetic influence, probably contributing to the variable outcomes of tuberculous meningitis. Interventions targeting tryptophan metabolism could improve outcomes of tuberculous meningitis.

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Introduction

Meningitis is the most severe manifestation of tuberculosis, resulting in death or neurological disability in more than 30% of adult patients.[1–3] The host immune system contributes to the poor outcome of tuberculous meningitis, either through inadequate killing of *Mycobacterium tuberculosis*, or through an inappropriate inflammatory response leading to tissue damage (immunopathology).[2] Cellular metabolism can help to determine the function of immune cells, and analysis of cerebrospinal fluid (CSF) metabolites could help unravel underlying biological mechanisms or establish the prognosis of tuberculous meningitis.

Metabolic studies in tuberculosis have examined a small number of metabolites, and most have focused on identification of host or bacterial metabolites as diagnostic markers.[4–6] We aimed to compare CSF and serum metabolomes of patients with tuberculous meningitis with those of controls with negative *M tuberculosis* culture, and assess the link between metabolite concentrations and mortality.

Methods

Study design and participants

This was a prospective observational cohort study done at the Hasan Sadikin Hospital (Bandung, Indonesia). Study participants were selected from a cohort of patients older than 14 years with suspected meningitis.[3] Eligible patients had definite tuberculous meningitis,[7] defined as positive CSF *M tuberculosis* culture or PCR, or probable tuberculous meningitis, defined as clinically suspected disease with at least five leucocytes per µL of CSF, and a

Research in context

Evidence before this study

We searched PubMed for articles published up to June 16, 2017, by title and abstract without language restrictions using the terms "tuberculous meningitis" or "tuberculosis" or "TB", with either "meningitis" or "central nervous system" (as Domain), combined with "metabolism" or "metabolite" (Determinant), combined with "survival", "mortality", "course", "prognosis", or "outcome" (Outcome). We did a second search without Outcome terms to increase sensitivity. We screened 55 articles, six of which contained information on metabolism in tuberculous meningitis. Glucose is low in the cerebrospinal fluid (CSF) of patients with tuberculous meningitis and associated with increased mortality in multiple studies. Other carbohydrates including lactate are increased and have been associated with greater mortality in one study, and a selection of aminoacids and lipid mediators are elevated in CSF of patients with tuberculous meningitis. Data on the relationship between metabolism and mortality, and possible targets for host-directed therapy are lacking, as an extensive metabolomics search has not yet been done.

Added value of this study

To our knowledge, this is the first study examining the CSF and serum metabolome of tuberculous meningitis in relation to patient mortality, using separate patient cohorts and a systems biology approach. Concentrations of many CSF metabolites showed a gradient, and were lowest in control participants, intermediate in patients with tuberculous meningitis who survived, and highest in those infected patients who later died. However, CSF tryptophan showed a unique pattern, with extremely low values in patients who survived. We validated this finding in a second patient cohort, and identified a genetic correlate of CSF tryptophan concentrations in tuberculous meningitis, which strongly predicted mortality in a third group of patients, supporting a causal role for tryptophan metabolism in outcomes of tuberculous meningitis.

Implications of all the available evidence

Tuberculous meningitis severely disturbs a much wider range of cerebral metabolic pathways than previously thought. Tryptophan metabolism—which affects *Mycobacterium tuberculosis* growth and CNS inflammation—is strongly related to survival of patients with tuberculous meningitis, providing possible targets for host-directed therapy. Further studies should target candidate genes identified through genetic analysis (eg, in experimental models), and examine tryptophan metabolism in relation to mortality of other cerebral infections.

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CSF to blood glucose ratio less than 0·5.[3] HIV infection, which strongly affects host cellular responses and increases mortality of tuberculous meningitis, was an exclusion criterion for this study (see appendix 1, p 2 for an overview of patient cohorts in the study). Oral consent to be included in the study, for storage of surplus sample, and to obtain follow-up data was obtained from patients or close relatives of patients who were unconscious. Control participants were individuals who had a lumbar puncture because of suspected CNS infection or subarachnoid bleeding but who showed no abnormalities on routine CSF examination (<5 leucocytes per µL, glucose ratio ≥0·5) and negative *M tuberculosis* culture.

Ethical approval was obtained from the Ethical Committee of Hasan Sadikin Hospital, Faculty of Medicine of Universitas Padjadjaran, Bandung, Indonesia.

Procedures

We determined the CSF and serum metabolome in a discovery cohort of 33 patients with tuberculous meningitis, of whom 17 died while in hospital and 16 survived during follow-up, and 22 controls without meningitis. Concentrations of metabolites were compared between patients and controls, and (among patients only) linked to survival. A second group of 101 patients with tuberculous meningitis and 17 controls was used for validation of the relationship between the CSF metabolome and survival. Genome-wide single nucleotide polymorphism (SNP) typing was done in 130 patients with tuberculous meningitis from the original discovery and validation cohorts to identify quantitative trait loci for individual CSF metabolites. Associations between identified genetic loci and patient survival were then validated in a third group of 285 tuberculous meningitis patients (the genetic validation cohort).

See Online for appendix 1

According to routine care, all patients with suspected meningitis underwent lumbar puncture before starting antimicrobial or corticosteroid treatment. Patients diagnosed with tuberculous meningitis underwent systematic clinical and CSF characterisation at the time of diagnosis, and survival was monitored prospectively for 1 year.

Gene Xpert MTB/RIF has only been used since 2015 and drug resistance testing was otherwise not routinely available in our study setting. Tuberculous meningitis was treated with a combination of rifampicin (unless otherwise indicated 450 mg, corresponding to about 10 mg/kg), isoniazid (300 mg), ethambutol (750 mg), and pyrazinamide (1500 mg) for 6 months according to Indonesian guidelines. Patients were given adjunctive dexamethasone according to the internationally accepted 6-week tapering regimen, starting at 0·3 mg/kg for grade I and 0·4 mg/kg for grade II or III tuberculous meningitis;[8] patients were switched to an equivalent dose of oral prednisolone in cases of early discharge.

CSF and serum metabolomics

CSF samples were centrifuged at 3000 rpm for 15 min and the supernatant was stored at –80°C. Serum was

collected after centrifugation of peripheral blood at 3000 rpm for 15 min. Samples were frozen and thawed once before metabolomics analysis. 425 metabolites were measured in serum and CSF using four liquid chromatography tandem mass spectrometry methods (see appendix 1, p 1 for more detail).[9,10]

For the **Kyoto Encyclopedia of Genes and Genomes pathway database** see http://www.genome.jp/kegg/pathway.html

For the **Small Molecule Pathway Database** see http://smpdb.ca

Quantification of CSF tryptophan and CSF and serum albumin

CSF tryptophan in the original and validation cohorts was quantified by ultra-performance liquid chromatography (see appendix 1, p 1 for more detail). The acquired ultra-performance liquid chromatography data showed good correlation with the original liquid chromatography-mass spectrometry data (Spearman's rho=0·95, p<0·0001). CSF and serum albumin were measured to determine the CSF to serum albumin ratio (normal range 0·005–0·008 for individuals aged 15–60 years;[11] see appendix 1, p 1 for more detail).

Metabolomic data analysis

Liquid chromatography-mass spectrometry and ultra-performance liquid chromatography data were analysed after log transformation. Values under the lower limit of detection of liquid chromatography-mass spectrometry were replaced with half of the metabolites' lower limit of detection in the specific matrix (CSF or serum). Pathway analysis was done on log-transformed, mean-centred data for metabolites with less than 50% missing values using MetaboAnalyst version 3.0,[12] which performs topological analysis with relative betweenness centrality applying the GlobalTest algorithm[13] to test for association of metabolite concentrations with diagnosis. Metabolites discovered in at least one sample were uploaded as a reference metabolome.

For the **GTEx database** see https://www.gtexportal.org/home/

For more on **Gene Set Enrichment Analysis** see http://software.broadinstitute.org/gsea/index.jsp

All other analyses were done in R version 3.2.2. Visualisation was achieved by principal component analysis on centred unscaled data using the R package prcomp. Comparison of metabolites was done using the R package limma. Figure colour gradients are based on uncorrected p values and tables show false discovery rates after applying the Benjamini-Hochberg procedure to correct for multiple testing. Survival analyses were done using the R paackage survival and visualised with the R package survminer. Differences between tryptophan strata were evaluated using a log-rank test. Multivariate survival analysis was done using Cox regression after log transformation of skewed continuous variables as indicated. Other R packages used were openxlsx, dplyr, reshape2 (for data handling), and tableone (for data representation), and all graphs were visualised using ggplot2 and enhanced by cowplot. Patients with negative CSF culture, who had received dexamethasone or antituberculosis drugs before lumbar puncture, who were infected with rifampicin-resistant strains, or who had received high-dose rifampicin were excluded from sensitivity analyses.

Transcriptional analyses

The tryptophan pathway was established based on the Kyoto Encyclopedia of Genes and Genomes (map 00380) and the Small Molecule Pathway Database (SMP00063), and all genes coding for enzymes in these reactions were included. For brain expression, data were available for five patients with tuberculous meningitis versus four patients deceased because of head injury (GSE23074), all from southern India.[14] Data were quantile-normalised, filtered for positive expression, log transformed, and analysed using the R package limma for association with patient group.

Tryptophan quantitative trait loci mapping and genotype-dependent survival analysis

Genotyping, quality control, and imputation was done as described in appendix 1 (p 1). Log-transformed, normalised concentrations of tryptophan were then mapped to the 4751257 variants that passed quality control (R^2>0·3 and minor allele frequency ≥0·1) using a linear regression model corrected for age and sex. We defined a threshold for suggestive genome-wide significance (p<0·00001). We calculated a prognostic index[15] using the linear component of the Cox model, as in prognostic index=$\beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_p x_p$, where x_1 is the genotype data in dosages and β_1 results from fitting the Cox proportional model. The genetic patient cohort was split into two groups according to the median of the prognostic index (low and high), and patients were evaluated for survival with a log-rank test. Finally, a prognostic index was used as a continuous covariate in multivariate Cox regression including possible confounders.

We used different approaches to examine the role of identified quantitative trait loci. First, identified quantitative trait loci were examined for possible effects on gene expression using the Gtex database. Second, genes neighbouring quantitative trait loci were extracted and expression was examined using Gene Set Enrichment Analysis in different human tissues and cell lines (deposited in the Human tissue compendium[16]). Finally, we used brain gene expression data[14] to assess the differential expression of genes near quantitative trait loci in patients with tuberculous meningitis compared with patients who had died from head injury.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

For the discovery metabolomics study, we included 33 patients with tuberculous meningitis with positive CSF culture between March 12, 2009, and Oct 27, 2013, including 16 who survived and 17 who died while in

hospital. We also included 22 individuals with negative *M tuberculosis* culture and normal routine CSF characteristics as controls (for an overview of the cohorts see appendix 1, p 2).[3]

Patients presented with severe disease, often with lowered consciousness, cranial nerve palsy, or paresis of one or more limbs (table 1). 425 metabolites were measured in serum and CSF. Quality and coverage of the metabolomics data was high, and routinely measured CSF glucose concentration was correlated with glucose concentration measured by liquid chromatography-mass spectrometry (Spearman's rho=0·986; p<0·0001). The CSF sample of one patient with tuberculous meningitis with aberrant measurements on the hydrophilic interaction chromatography-positive platform was removed from subsequent analyses. Of 425 annotated metabolites, 348 (82%) were detected in CSF and serum, three (1%) in CSF only, and 42 (10%) in serum only (appendix 2).

Patients with tuberculous meningitis had a distinct CSF metabolome compared with control participants, as shown by principal component analysis (figure 1). One patient with tuberculous meningitis and miliary tuberculosis who had no leucocytes in CSF clustered with the control group; we did not exclude this patient from the tuberculous meningitis group for the analysis as no technical anomaly was detected. Of 351 metabolites detected in CSF, 250 (71%) were higher and 18 (5%) were lower in patients with tuberculous meningitis than they were in controls (with a false discovery rate threshold of <0·05; figure 1). Metabolite concentrations showed large variation, but were on average 12-times higher in serum than in CSF (appendix 1, p 3). The median CSF to serum albumin ratio was 0·050 in patients with tuberculous meningitis, suggesting that the blood–CSF barrier was severely disrupted, compared with 0·0062 in controls (appendix 1, p 3). The difference in average relative abundance of metabolites between CSF and serum was smaller in patients (6·2-times) than in controls (32·2-times). CSF metabolites across the whole range of quantity and mass showed substantial differences between patients and controls (appendix 1, p 3), with 58 (97%) of 60 known metabolic pathways being affected (appendix 1, p 10). The serum metabolome showed more subtle differences between patients and controls (figure 1), with only five (1%) of 397 metebolites being significant (false discovery rate <0·05; figure 1).

We next compared metabolite profiles of patients with tuberculous meningitis who died while in hospital (n=17, median time to death 4 days [IQR 3–7]) and those who were discharged alive (n=15, after exclusion of the sample with aberrant measurements). Patients who survived were discharged after a median of 25 days (IQR 21–29), and one additional outpatient death was recorded at day 89. None of the serum and CSF metabolites reached a false discovery rate threshold of less than 0·05 for comparison between survivors and non-survivors. At an ordinary significance level (p<0·05), 33 (8%) of 390 metabolites detected in serum differed between non-surviving and surviving patients with tuberculous meningitis, but only one of these (urate) also differed between patients and controls. At an ordinary significance level (p<0·05), 28 (8%) of 351 metabolites detected in CSF differed between non-survivors and surviving patients with tuberculous meningitis (figure 2). 25 (90%) of 28 metabolites were higher in non-surviving patients than in surviving patients with tuberculous meningitis. Of these 25 CSF metabolites, 13 (52%) were higher in the original comparison of patients with tuberculous meningitis compared to controls. One of these 13 metabolites, the eicosanoid leukotriene B4—a downstream chemoattractant of *LTA4H* that has been studied in relation to tuberculous meningitis survival—was lowest in controls, 11-times higher in patients with tuberculous meningitis who survived, and 30-times higher in patients who died. Two metabolites, glucose and inositol, followed the reverse pattern. For example, glucose was highest in controls, 2·5-times lower in patients with tuberculous menininginitis who survived, and 4-times lower in those who died. Three CSF metabolites (putrescine, cytidine, and tryptophan) followed a pattern in which survivors had lower concentrations than both controls and non-survivors. CSF tryptophan, which had the largest difference between groups (figure 2), was 31-times lower in survivors than in controls, and 9-times lower in survivors than non-survivors (unadjusted p=0·00043).

See Online for appendix 2

Unlike many other metabolites, CSF tryptophan was not associated with CSF to serum albumin ratio (a marker of disruption to the blood–CSF barrier) (Pearson's r^2=0·015), CSF polymorphonuclear cell count (Pearson's r^2=0·058), or CSF mononuclear cell count (Pearson's r^2=0·019), suggesting that CSF tryptophan concentrations instead reflect metabolism in the brain itself (appendix 1, p 4; for correlations for all other metabolites see appendix 2). Serum tryptophan concentrations were 1·3-times higher in patients who survived than they were in those who died (p=0·055; appendix 2).

We tested the association between CSF tryptophan concentrations and patient survival in a separate group of 101 consecutively recruited patients with tuberculous meningitis (recruited between July 2, 2014, and April 6, 2016), 67 (66%) of whom were culture-confirmed and 17 control participants (table 1). The concentration of CSF tryptophan, quantified using ultra-performance liquid chromatography, was 0·20 µM in survivors, 1·11 µM in non-survivors, and 2·08 µM in controls (p<0·0001 for 30-day survival; appendix 1, p 5). Low CSF tryptophan concentration predicted survival in Cox regression (hazard ratio [HR] for mortality 0·73; 95% CI 0·64–0·83; p<0·0001, for each halving in CSF tryptophan concentration; figure 2). The HR was not affected by correction for sex, age, Glasgow Coma Scale, CSF neutrophils, CSF mononuclear cells, and rs17525495

	Metabolomics discovery cohort			Tryptophan validation cohort		Genetic validation cohort
	Controls (n=22)	Tuberculous meningitis survivors (n=15*)	Tuberculous meningitis non-survivors (n=17)	Controls (n=17)	Tuberculous meningitis (n=101)	Tuberculous meningitis (n=285)
Clinical features						
Sex						
Male	10 (45%)	11 (73%)	11 (65%)	9 (53%)	62 (61%)	153 (54%)
Female	12 (55%)	4 (27%)	6 (35%)	8 (47%)	39 (39%)	132 (46%)
Age, years	34 (22–25)	27 (25–34)	27 (22–30)	39 (22–39)	30 (21–38)	29 (22–37)
Tuberculous meningitis grade						
I	NA†	1 (7%)	0/16	NA	3/99 (3%)	30/258 (12%)
II	NA†	13 (87%)	8/16 (50%)	NA	82/99 (84%)	195/258 (76%)
III	NA†	1 (7%)	8/16 (50%)	NA	14/99 (14%)	33/258 (13%)
Temperature, °C	37·0 (36·7–37·5)	37·6 (36·9–38·2)	37·6 (37·2–37·9)	37·1 (36·8–37·5)	37·8 (37·0–38·3)	37·5 (36·8–38·0)
Glasgow Coma Scale	14 (13–15)	13 (12–14)	12 (11–13)	15 (14–15)	13 (11–14)	14 (12–15)
Seizures present	4 (18%)	0	2/16 (13%)	2 (12%)	5/98 (5%)	17/246 (7%)
Motor abnormalities present	14 (64%)	6 (40%)	9/16 (56%)	8 (47%)	59/99 (60%)	127/241 (53%)
Cranial nerve palsy present	13/21 (62%)	8 (53%)	14 (82%)	9 (53%)	69/100 (69%)	161/258 (62%)
Cerebrospinal fluid features						
Leucocytes, cells per µL	2 (0–2)	99 (50–306)	54 (15–275)	2 (1–2)	236 (124–443)	138 (49–308)
Neutrophils, cells per µL	0 (0–1)	26 (14–66)	38 (6–171)	1 (0–1)	78 (25–186)	27 (8–89)
Mononuclear cells, cells per µL	1 (0–2)	84 (29–201)	47 (11–118)	1 (1–1)	136 (91–210)	74 (29–165)
Protein, mg/dL	34 (19–47)	291 (126–357)	216 (143–371)	27 (20–33)	173 (125–355)	190 (102–373)
Cerebrospinal fluid to blood glucose ratio	0·64 (0·56–0·70)	0·21 (0·13–0·29)	0·12 (0·08–0·17)	0·64 (0·56–0·68)	0·17 (0·10–0·25)	0·21 (0·12–0·33)
Mycobacterium tuberculosis culture positive	0	15 (100%)	17 (100%)	0	67 (66%)	138/281 (49%)
Blood features						
Haemoglobin, g/dL	12·0 (9·40–13·8)	11·9 (10·1–13·8)	12·4 (11·2–13·3)	11·0 (9·0–12·8)	12·4 (10·5–13·7)	12·1 (10·5–13·4)
Leucocytes, x 10^9/L	10·1 (7·6–12·6)	10·0 (8.8–11·3)	12·6 (8·5–15·8)	7·5 (5·2–8·7)	11·0 (8·8–13·7)	11·0 (7·9–14·3)
Thrombocytes, x 10^9/L	278 (136–416)	293 (192–351)	346 (301–400)	254 (168–305)	298 (227–378)	290 (216–377)
Outcomes						
Length of hospital stay, days	16 (12–17)	25 (21–29)	4 (3–7)	11 (4–20)	20 (11–23)	16 (8–22)
Alive at discharge	NA	15 (100%)	0	NA	72/100 (72%)	187/242 (77%)
Outcome at day 30						
Alive	NA	14 (93%)	0	NA	69 (68%)	199 (70%)
Deceased	NA	0	17 (100%)	NA	29 (29%)	74 (26%)
Lost to follow-up	NA	1 (7%)	0	NA	3 (3%)	12 (4%)
Outcome at day 180						
Alive	NA	12 (80%)	0	NA	52 (51%)	153 (54%)
Deceased	NA	1 (7%)	17 (100%)	NA	40 (40%)	99 (35%)
Lost to follow-up	NA	2 (13%)	0	NA	9 (9%)	33 (12%)

Data are n (%) or median (IQR). Quantitative trait loci were identified in the metabolomics discovery and tryptophan validation cohort combined (excluding three patients without genotype data). Data are missing for some patients, as indicated. NA=not applicable. *One patient was excluded from the analysis and table because of aberrant liquid chromatography-mass spectrometry results. †Eight control patients in the metabolomics discovery cohort were diagnosed with primary CNS disease (dementia, epilepsy, meningoencephalitis, neurolupus, space-occupying lesions, or stroke), 11 had CNS manifestations of systemic disease (active pulmonary or miliary tuberculosis, advanced heart or kidney disease, or metabolic encephalopathy), and three had no final diagnosis.

Table 1: **Patient characteristics**

LTA4H promotor polymorphism,[3,17,18] either alone or in combination (table 2). This effect was not different in sensitivity analyses that excluded patients who were not culture-confirmed (n=33), patients who had received treatment before lumbar puncture (nine), patients who had received high-dose rifampicin (n=21), patients who had not received dexamethasone (four), and one patient with rifampicin-resistant tuberculous meningitis (data not shown).

Based on the metabolomic data of the discovery cohort, cerebral tryptophan metabolism appeared to be one of the most upregulated pathways in patients with tuberculous meningitis compared with controls (false discovery rate $<10^{-19}$; appendix 1, p 10). Although CSF

tryptophan concentrations were lower in patients with tuberculous meningitis compared with controls, downstream metabolites in the kynurenine pathway were 4–60-times higher in CSF samples of patients with tuberculous meningitis than in controls, although none of the downstream metabolites showed a significant association with survival (appendix 1, p 6; appendix 2). We analysed publicly available gene expression data from brain autopsies of five patients with tuberculous meningitis and four patients with traumatic brain injury for gene expression in the kynurenine pathway.[14] Indoleamine 2,3-dioxygenase (*IDO1*), which is expressed in astrocytes and neurons[19] and codes for the rate-limiting enzyme that converts tryptophan to L-formylkynurenine, showed greater expression in patients with tuberculous meningitis than in patients with brain trauma (35-times upregulation, p=0·008; α=0·05/10 for the ten genes tested; appendix 1, p 7), suggesting that increased tryptophan metabolism might lead to lower CSF tryptophan in tuberculous meningitis.

We mapped quantitative trait loci for CSF tryptophan concentrations using genome-wide SNP genotype data (available for 130 [98%] of 133 patients with available CSF tryptophan concentrations from the discovery and validation cohorts; appendix 1, p 2) to assess whether host genetic variation plays a part in the regulation of the amount of tryptophan in CSF. No single SNP showed genome-wide significance for CSF tryptophan concentrations, but we identified 11 independent loci that showed suggestive associations with CSF tryptophan levels (p<0·00001; figure 3; table 3).

These 11 identified quantitative trait loci, along with age and sex, were used to generate a composite prognostic index to predict survival among patients with tuberculous meningitis. As expected, this score strongly predicted survival among the 130 patients we used for identification of the quantitative trait loci (figure 3). The relevance of the prognostic index was validated in a separate group of HIV-negative patients with tuberculous meningitis, for whom no CSF tryptophan concentrations were available (appendix 1, p 2). The prognostic index composed of the 11 tryptophan quantitative trait loci also strongly predicted patient survival in this independent cohort (p=0·023 on log-rank test for 180-day survival, n=285), and this predictive power was further improved by including age and sex in the model (p=0·0054; figure 3), although age and sex alone did not predict survival (p=0·823). The prognostic index showed a similar effect in sensitivity analysis restricted to 166 bacteriologically confirmed cases (p=0·021), and after correction for possible confounders (included in the multivariate analysis presented in table 2).

Finally, we examined the possible biological relevance of the 11 tryptophan quantitative trait loci. Only one tryptophan quantitative trait locus was known to influence expression of a gene, *WBP4*, the expression of which was affected in skeletal muscle (p<0·0001) and tibial nerve (p=0·0006). As a second approach to prioritise causal genes at tryptophan quantitative trait loci, we extracted publicly available expression data for annotated genes located within a 1-Mb *cis*-window of the 11 tryptophan quantitative trait loci in different human tissues and cell lines. Unsupervised clustering analysis of the 54 (77%) of 70 annotated genes with data available showed that the tryptophan quantitative trait loci were mainly expressed in immune cells and brain tissues (appendix 1, p 9). In brain autopsy data of patients with tuberculous meningitis,[14] 17 (24%) of 70 annotated genes showed differential expression compared to patients who died of head injury (meeting a cutoff of p<0·05; table 3). Pathway analysis for these genes did not reveal enrichment for any one pathway (data not shown), but this selection emphasised the role of immune genes (*TRIL*, *NAP1L1*, and *OSBPL8*), metabolic enzymes (*MAG11*), and brain-specific genes (*WBP4*, *GAS7*, and

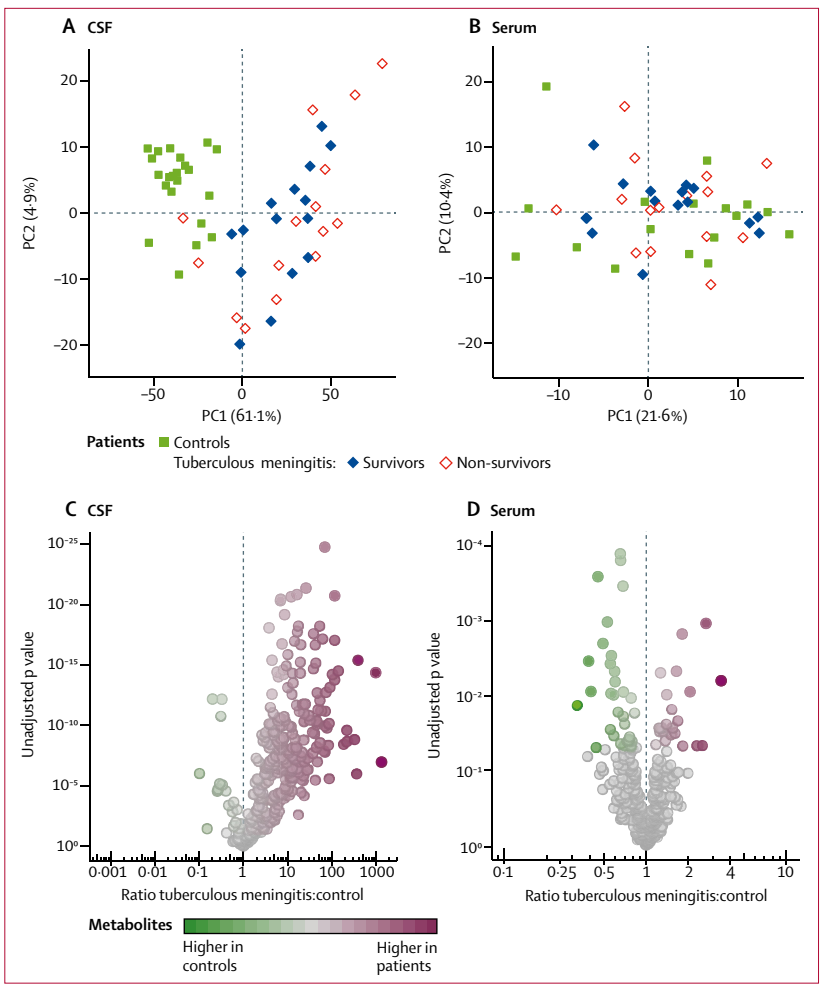

Figure 1: **CSF and serum metabolome in patients with tuberculous meningitis and controls**
Principal component analysis for CSF (A) and serum (B) with proportion of variance per principal component indicated between brackets. Volcano plots for individual metabolites in CSF (C) and serum (D). Colours indicate strength of association. Metabolites that show no difference between groups (uncorrected p>0·05) are grey. CSF=cerebrospinal fluid. PC=principal component.

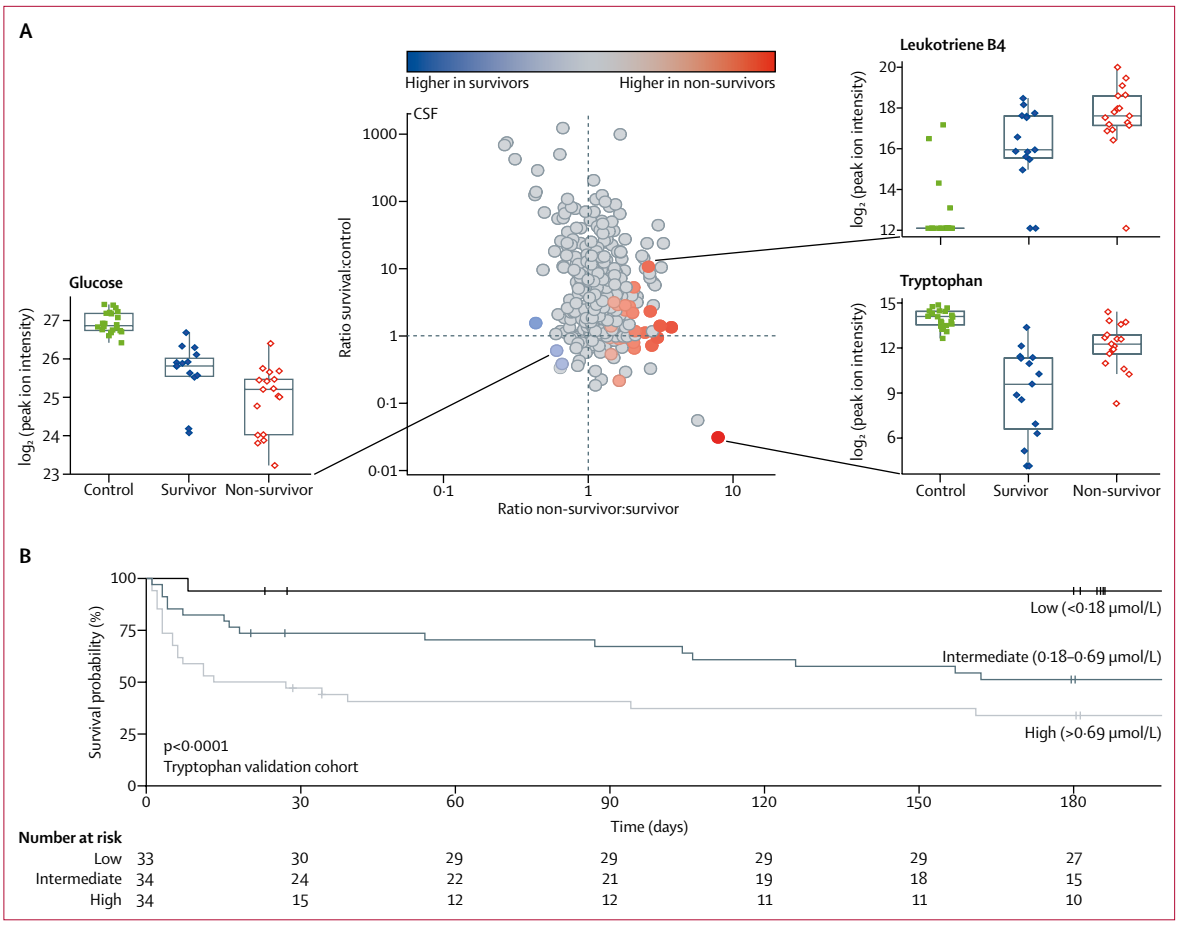

Figure 2: **CSF metabolome as a determinant of survival in patients with tuberculous meningitis**
(A) Individual metabolites in CSF with ratio between tuberculous meningitis survivors and non-survivors, and ratio between tuberculous meningitis survivors and controls. Colours indicate strength of association; metabolites that do not show differences between groups (uncorrected p>0·05) are grey. The three subplots show metabolite concentrations according to patient category. The y axis shows the 2-log of the relative abundance of metabolite ions as chromatographic peaks (peak ion intensity). Leukotriene B4, glucose, and tryptophan were chosen as relevant metabolites representing three different quadrants of the plot. (B) Kaplan-Meier plot of patient survival in the tryptophan validation cohort, according to CSF tryptophan concentrations, as divided in the following tertiles: low (<0·18 µmol/L), intermediate (0·18–0·69 µmol/L), and high (>0·69 µmol/L). CSF=cerebrospinal fluid.

	Tryptophan validation cohort (n=100)		Genetic validation cohort (n=235)	
	HR (95% CI)	p value	HR (95% CI)	p value
Sex, male	0·86 (0·42–1·72)	0·669	NA*	NA*
Age, per 10-year increase	1·06 (0·80–1·40)	0·691	NA*	NA*
Glasgow Coma Scale, per point increase	0·76 (0·66–0·88)	0·00026	0·80 (0·72–0·88)	<0·0001
CSF neutrophils, per ten-times increase	1·70 (0·86–3·36)	0·127	1·53 (1·10–2·12)	0·011
CSF mononuclear cells, per ten-times increase	0·32 (0·14–0·70)	0·0044	0·75 (0·50–1·15)	0·188
LTA4H genotype†, CT *vs* CC	0·76 (0·36–1·59)	0·460	1·06 (0·65–1·71)	0·823
LTA4H genotype†, TT *vs* CC	0·35 (0·07–1·62)	0·177	0·72 (0·30–1·69)	0·446
CSF tryptophan, per two-times decrease	0·74 (0·63–0·87)	0·00033	NA	NA
Prognostic index	NA	NA	NA	0·0083

Patients with complete data were as follows: tryptophan validation cohort (n=100, with 39 events), genetic validation cohort (n=235, with 81 events); for multivariate analysis, one patient was excluded from the tryptophan validation cohort and 50 were excluded from the genetic validation cohort because of missing Glasgow Coma Scale or *LTA4H* genotype data. CSF cell counts were analysed after $\log_{10}(x+1)$ transformation. Prognostic index was included as a continuous variable. HR=hazard ratio. NA=not applicable. CSF=cerebrospinal fluid. *Age and sex were included in the prognostic index together with the 11 tryptophan quantitative trait loci. †rs17525495.

Table 2: **Multivariate Cox regression for 180-day mortality in the tryptophan validation cohort and independent genetic validation cohort**

CHN2) in determining tryptophan concentrations in CSF of patients with tuberculous meningitis.

Discussion

To our knowledge, this is the first study comparing the CSF and serum metabolome of patients with tuberculous meningitis and controls without meningitis, linking metabolite concentrations to patient mortality. CSF tryptophan concentration was identified as a strong predictor of mortality, and this finding was validated in a second patient cohort. Furthermore, by using genome-wide SNP data we identified 11 quantitative trait loci associated with CSF tryptophan concentrations, and found that these quantitative trait loci were predictive of patient survival in a third cohort of patients with tuberculous meningitis. Collectively, our data showed that cerebral tryptophan metabolism is important for the outcome of tuberculous meningitis.

We also believe this is the first study to examine the outcome of tuberculous meningitis using a combination of metabolomic and genomic approaches. In accordance with earlier studies, we found lower CSF glucose and higher lactate concentrations,[20] and increased amounts of aminoacids[21] in CSF of patients with tuberculous meningitis compared with controls. We also confirmed that glucose was lowest in patients who subsequently died. We focused on tryptophan because of its distinct pattern and biological relevance for tuberculosis. The combination of lower CSF tryptophan concentrations, higher concentrations of downstream kynurenine metabolites, and upregulated *IDO1* in brain autopsy mRNA expression profiles suggests that *M tuberculosis* brain infection leads to increased cerebral tryptophan metabolism. Based on these findings, we concluded that an individual's genetic makeup determines the response in tryptophan metabolism if *M tuberculosis* invades the brain to cause meningitis, with lower CSF tryptophan associated with reduced mortality.

Low CSF tryptophan metabolite concentrations have previously been found in bacterial meningitis,[22] trypanosomiasis,[23] rabies,[24] and cerebral malaria.[25] However, to our knowledge, CSF tryptophan has never been reported in relation to survival of patients with CNS infection. Several possible explanations exist for how tryptophan and kynurenine metabolism might affect the outcome of tuberculous meningitis. First, activated macrophages could inhibit *M tuberculosis* growth through activation of *IDO1*, with depletion of tryptophan as an energy source for mycobacteria.[26] Second, tryptophan and its downstream metabolites affect T-cell responses and inflammation. For instance, the aryl hydrocarbon receptor senses kynurenine,[27] which is engaged in *M tuberculosis*-infected macrophages and crucial for several innate immune responses.[28] Finally, certain kynurenine metabolites, such as kynurenic acid and quinolinic acid, are involved in neuroprotective and neurodamaging responses.[19]

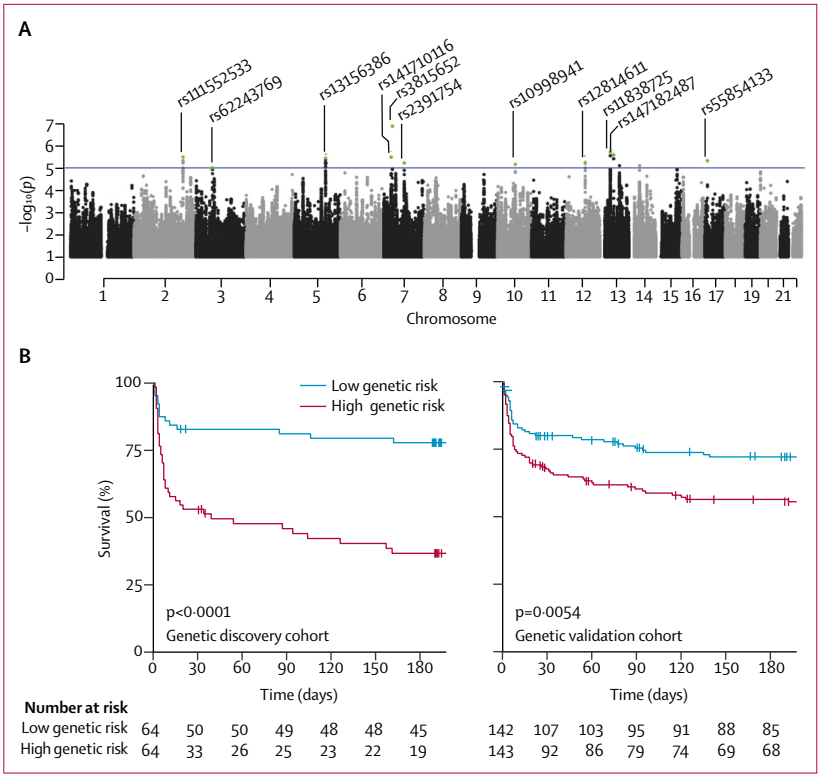

Figure 3: **CSF tryptophan quantitative trait loci in relation to survival of patients with tuberculous meningitis**
(A) Manhattan plot for association of CSF tryptophan concentrations with single nucleotide polymorphisms on 22 somatic chromosomes. The horizontal line depicts the threshold chosen to call suggestive associations ($p<10^{-5}$), with the accession number (rsID) of 11 independent single nucleotide polymorphisms associated with tryptophan concentrations. (B) Kaplan-Meier plots using prognostic index including these 11 tryptophan quantitative trait loci, age, and sex, predicting survival of patients with tuberculous meningitis in the genetic discovery cohort and genetic validation cohort. CSF=cerebrospinal fluid.

Therefore, the relative abundance and balance of tryptophan and kynurenine metabolites is likely to affect the outcome of tuberculous meningitis. Notably, compared to tryptophan, individual downstream metabolites showed smaller differences between survivors and non-survivors, probably because tryptophan is metabolised via different pathways.

We found a genetic correlate of CSF tryptophan concentration in tuberculous meningitis that predicted survival in an independent cohort, which suggested the intrinsic ability to upregulate tryptophan metabolism in response to *M tuberculosis* infection affects the balance between bacterial clearance and immunopathology in tuberculous meningitis. As there are no Indonesian specific SNP reference panels available for imputation we should be cautious about inferring causality of identified variants. Nevertheless, further analysis of CSF tryptophan quantitative trait loci identified several candidate genes with immune, metabolic, or brain-specific functions, which could be targets for further study (eg, in experimental models). Tryptophan metabolism is an attractive target for adjunctive therapy. Treatment with interferon-α successfully increased peripheral blood but

	Chromosome	Position	Reference allele (A)	Minor allele (a)	In tuberculous meningitis cohort					Association with CSF tryptophan concentration in tuberculous meningitis*		Biological plausibility	
					Minor allele frequency	AA	Aa	aa	Imputation accuracy (R^2)	p value	β	Nearest gene	Genes indicated by differential expression analysis†
rs111552533	2	192 996 515	C	T	0·42	47	63	20	0·87	$3·21\times10^{-6}$	1·26	*TMEFF2*	*SDPR*
rs62243769	3	65 949 227	C	G	0·40	48	60	22	0·95	$9·85\times10^{-6}$	−1·14	*MAGI1*	*MAGI1*
rs13156386	5	123 017 888	A	G	0·27	72	50	8	0·75	$3·61\times10^{-6}$	1·60	*CSNK1G3*	*CEP120*
rs141710116	7	80 867 056	T	C	0·23	72	56	2	0·75	$5·91\times10^{-6}$	−1·94	*SEMA3C*	*HGF, SEMA3C*
rs3815652	7	33 913 404	T	C	0·32	58	63	9	0·66	$1·31\times10^{-7}$	1·83	*BMPER*	*BMPER, BBS9*
rs2391754	7	29 403 188	T	C	0·20	87	38	5	0·47	$3·26\times10^{-6}$	1·73	*CHN2*	*TRIL*
rs10998941	10	71 495 726	T	G	0·44	39	65	26	0·87	$6·89\times10^{-6}$	1·22	*COL13A1*	*TYSND1*
rs12814611	12	76 384 849	T	C	0·43	37	74	19	0·95	$5·61\times10^{-6}$	1·28	*PHLDA1*	*OSBPL8, BBS10, NAP1L1, PHLDA1*
rs11838725	13	41 671 085	T	C	0·21	77	50	3	0·62	$1·91\times10^{-6}$	−1·89	*WBP4*	*RGCC, WBP4, FOXO1*
rs147182487	13	54 547 574	T	C	0·13	36	94	0	0·47	$2·56\times10^{-6}$	2·35	*LINC00558*	..
rs55854133	17	10 497 745	A	G	0·15	98	29	3	0·70	$4·75\times10^{-6}$	1·87	*MYHAS*	*GAS7*

AA, Aa, and aa genotypes are listed, where A represents the major allele and a the minor allele. CSF=cerebrospinal fluid. SNP=single nucleotide polymorphism. *Linear regression analysis was used for quantitative trait loci mapping upon correcting for age and sex. †Based on differential expression analysis in tuberculous meningitis versus brain injury patients data (using data from Kumar and colleagues[14]), genes located within 1 Mb cis window of 11 tryptophan quantitative trait loci (n=70 for the 11 quantitative trait loci) that might contribute to tryptophan concentration in CSF of patients with tuberculous meningitis were identified.

Table 3: **CSF tryptophan quantitative trait loci in tuberculous meningitis**

not CSF kynurenine concentrations,[29] and ingestion of a 15-aminoacid, trytophan-free mixture strongly reduced CSF tryptophan concentrations.[30]

Strengths of our study are the careful description and prospective follow-up of patients, the combination of metabolomics and genetics, and the validation of findings in two separate patient groups. Limitations of the metabolomic approach include the large but still restricted number of annotated metabolites, which form a fraction of the incompletely characterised human metabolome. Therefore, metabolites other than tryptophan that are not yet annotated could be better predictors for survival. Moreover, the discovery cohort included a relatively small number of culture-confirmed, HIV-negative patients, so further study is needed to discover metabolites with smaller effect sizes and to expand our findings to HIV-positive patients. We showed a genetic association with tryptophan concentration and mortality, but the observational nature of our study precludes statements on the causative mechanism. Further study is therefore needed to identify specific metabolic steps in cerebral tryptophan metabolism that are linked to immunopathology and patient survival. Also, confirmation of our results in a cohort with a different genetic background could validate the ability of specific quantitative trait loci in their ability to predict CSF tryptophan concentration and survival. Intervention studies should help establish if tryptophan metabolism can be used as a target for adjuvant treatment in tuberculous meningitis.

In summary, a low CSF tryptophan concentration strongly predicted patient survival, and no association was found between serum tryptophan and mortality. CSF tryptophan concentrations in patients with tuberculous meningitis were under genetic influence, and genetic loci correlating with CSF tryptophan concentrations also predicted survival in an independent patient group. Collectively, these data suggest that cerebral tryptophan metabolism is crucial for survival of tuberculous meningitis. Our findings provide possible new strategies for host-directed therapy (eg, pharmacological induction of tryptophan metabolism) for tuberculous meningitis. Our integrative approach of combining CSF metabolomics and genetics with routine patient characteristics and survival also holds promise for identification of other relevant biological pathways and targets for adjuvant therapy, both in tuberculous meningitis and in other CNS infections.

Contributors

AvL, CBC, MGN, and RvC designed the study. SD, THA, RR, and ARG supervised patient recruitment. JA, LC, and BA supervised patient sample flow. AvL, CR, and SD did patient data quality control. CBC oversaw metabolomics data acquisition and JA-P and CBC analysed and interpreted these data. MMV supervised ultra-performance liquid chromatography tryptophan measurement. RA-G, IR-P, YL, and VK did the genetic analysis. AvL and VACMK did other bioinformatic analyses. RAN and LABJ contributed to metabolic concepts. AvL and RvC wrote the first draft of the manuscript. All other authors provided input to the draft and approved the final version of the manuscript.

Declaration of interests

We declare no competing interests.

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