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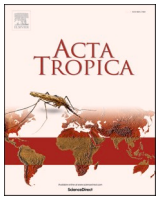

Cytokine levels in the severity of falciparum malaria: An umbrella review

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ABSTRACT

This study aimed to synthesise evidence comparing the levels of cytokines in severe falciparum malaria with those in uncomplicated malaria from available systematic reviews and meta- analyses. Relevant individual meta-analyses were searched in PubMed, Ovid, and Google Scholar, following the selection criteria specified for this umbrella review. The AMSTAR-2 tool was applied to grade the quality of the meta-analyses identified. The random-effects model was applied to recalculate the effect sizes of each included meta-analysis. Heterogeneity between meta-analyses was investigated with I^2 value. 95% predicting interval (PI) for the summary random-effects model was also made. In each meta-analysis identified, information on largest study's effect, the excess significance test, small study effects, and publication bias were addressed. This umbrella review included nine meta-analyses ($n = 12{,}674$) for nine unique cytokines (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, and TNF-α). Only one individual meta-analysis showed significantly higher levels of cytokine IL-1β (p: 0.009) amongst those with severe falciparum malaria compared to those with uncomplicated malaria. The 95% PIs did not show significance in any individual meta-analyses. Nine individual meta-analyses showed substantial heterogeneity, with I^2 tests ranging from 81% to 99%. Two independent meta-analyses (the IL-4 and IL-12) showed evidence of 'excess significant bias'. The meta-analysis of IL-1β only showed "Class III evidence", indicating that this cytokine was "suggestive" in contributing to those with severity of malaria in comparison to those with uncomplicated malaria. The remaining eight cytokines showed "Class IV evidence," indicating "weak" evidence on the impact of malaria severity.

In conclusion, the findings suggest that compared to uncomplicated malaria, pro-inflammatory cytokine IL-1β contributes to the development of severe falciparum malaria. Due to the limited level of evidence, further well-designed larger studies with multiple cytokines are needed to investigate cytokine levels as reliable biomarkers in malaria severity.

1. Introduction

Malaria transmission continues worldwide despite substantial reduction in 85 malaria endemic countries and territories. There is now an estimated 249 million cases globally with 5 million new cases reported in 2021 (WHO, 2023). *Plasmodium falciparum* is common in the tropical region and is attributed to the most serious form of the disease (WHO, 2012). Studies report that infants are more susceptible to severe malaria than adults (Farrington, 2017; White, 2022). This can be explained by the situation that infants' immune systems often clear pathogens more slowly than those of adults and older children and show biased regulatory reactions to the synthesis of regulatory Th1 and Th17 cytokines (White, 2002; Goriely, 2004).

An increase in peripheral blood cytokines and chemokines during an acute malaria infection contribute to the removal of the parasite, but is also likely to be the basis for many symptoms and physiological changes (Farrington, 2017). Cytokines are known to regulate the host's immune system as well as the inflammatory, trauma, and infection responses. Certain cytokines have an inflammatory effect, whereas other cytokines have an anti-inflammatory effect, which reduces inflammation and promotes healing (Dinarello, 2000). During the stage when malaria parasites are in the blood stream, the host's immune system releases an

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array of proinflammatory molecules including IL-1β, IL-6, IL-8, IL-12 (p70), INF-γ, and TNF, which plays a defining role in controlling parasite's growth and elimination (Popa and Popa 2021). Reliable biomarkers capable of predicting the progression of severe malaria would be a valuable addition to malaria control programs, especially in regions with weak health systems where access to quality diagnostic services is limited, and skilled clinical workforce are not available (Foko, 2022).

Numerous individual studies have examined the roles of pro- and anti-inflammatory cytokines in the development of severe malaria. Nevertheless, their results have been inconsistent. Furthermore, they are studies with small sample sizes. Findings of individual observational studies on the relationship between cytokines and severe malaria have been summarized by an increasing number of systematic reviews and meta-analyses. Systematic reviews are often used to quantitatively collate the results of numerous research studies to obtain a pooled estimate of the effect (Ranganathan and Aggarwal, 2020). It appears that there have been several systematic reviews that addressed the roles of particular cytokines in severe falciparum malaria including IL-1β (Mahittikorn et al., 2022), IL-6 (Wilairatana, 2022a), IL-12 (Wilairatana 2022b), amongst others.

Substantial variation as well as potential bias are limiting the evidence, even with reliable data and high-quality methodological procedures (Ioannidis, 2005). A comprehensive overview of the evidence could therefore be generated by combining information obtained from various meta-analyses. An umbrella review is a cluster that encompasses many reviews (Ioannidis, 2009), and it is most useful when numerous systematic reviews exist on a related topic, and they can be used for systematically integrating, evaluating, and aggregating the results of systematic reviews (Aromataris, 2015). Taken together, the current umbrella review aimed to synthesise evidence from multiple systematic reviews comparing the levels of cytokines in severe falciparum malaria excluding cerebral malaria with those in uncomplicated malaria from available systematic reviews and meta-analyses.

2. MATERIALS and methods

This umbrella review adhered to the Preferred Reporting Items for Overviews of reviews (PRIOR) checklist (Gates, et al. 2022) (Supplementary File 1). There was no involvement of patients in the current study. This study's protocol is available in PROSPERO (CRD42024525999).

2.1. Search strategy

Health-related databases of PubMed, Ovid, and Google Scholar were searched for relevant meta-analyses. During the search procedure, the following terms were used: "severe malaria" OR "anaemia" AND "plasmodium falciparum" AND "meta-analysis" OR "systematic review" AND "cytokines" OR "plasma cytokines" OR "serum cytokines" OR "interleukins" OR "IL-10″ OR "IL-4," OR "IL-12," OR "tissue necrosis factors," OR "TNF." Additionally, the federated search engine "Epistemonikos" (www.epistemonikos.org), that focuses specifically on research syntheses, was used. The list of references in relevant studies that might have been overlooked during a database search was identified through a manual search. Supplementary File 2 describes the search strategies.

2.2. Studies selection

The eligibility of studies was as described below.

1) An individual systematic review/meta-analysis (referred to as meta-analysis from here on) of observational studies, irrespective of geographical location, and number of included studies.
2) Compare those with severe falciparum malaria and those with uncomplicated malaria for outcome of cytokine levels. Any cytokines, not limited to IL-1β, IL-2, IL-4, TNF-α, were considered. Reviews that covered multiple malaria infections were included if they reported data on falciparum malaria patients separately.
3) If primary studies in any single meta-analysis enroled participants infected with *P. falciparum* or *P. vivax*, we considered the meta-analysis where data on falciparum malaria patients were reported separately or where the *P. falciparum* case was 60% or more.
4) Cytokine levels were expressed with mean and standard deviation (SD) or median and range in pg/mL). If data were reported in median and range, a conversion was made into mean and SD (Wan et al., 2014). For instance, if the data provided median (q1-q3), the formulas [mean = ((q1+m1+ q3)/3)] and [SD = ((q3-q1)/1.35)); where m = median were used to convert the data into mean and its SD.

The selection of studies was limited to publications in English from 1 January 1993 to 1 August 2024. Severe malaria and uncomplicated malaria were as defined in the primary meta-analysis studies. According to the WHO guideline (WHO, 2022), clinical manifestations of severe malaria include shock, pulmonary oedema, impaired consciousness, prostration, repeated convulsions, hypoglycemia, severe anaemia, acidosis, jaundice (bilirubin > 3 mg/DL) with parasite density > 100, 000/μL), renal impairment, and hyper-parasitaemia (parasite count > 10, 000/ μL). Meta-analysis of primary studies on participants diagnosed exclusively with cerebral malaria were not included in this umbrella review as this presentation has a different pathophysiology. However, meta-analysis of primary studies on participants diagnosed with cerebral malaria and non-cerebral malaria were found, it was considered if data on non-cerebral malaria were provided separately. Non-severe malaria was regarded as uncomplicated malaria unless otherwise stated. Meta-analysis studies with serum cytokines were not considered.

Meta-analysis studies that did not meet the above criteria were excluded. We excluded narrative reviews or systematic reviews that did not include quantitative synthesis.

2.3. Data collection

Two investigators (CN, AKB) individually performed data collection. For completeness, information was collected both from individual meta-analysis identified as well as primary studies included in the respective individual meta-analysis. Collected data from individual meta-analysis were first author, year of publication, number of included primary studies, malaria species, age group of participants, number of participants in the two groups, summary mean difference (MD) along with SDs (or median and range), and the I^2 statistics, the Q test and tests for publication bias (Egger's test) (Egger, 1997). The I^2 value indicates percentage of variations across studies due to heterogeneity. For the Q test, a P value > 0.10 indicates little heterogeneity, while a P value < 0.10 is a presence of heterogeneity (Higgins et al., 2023). For individual primary studies whenever needed to update the existing meta-analysis, the same information was collected, along with MDs and its SDs in the two groups. Any differences of data between the two investigators were settled by discussion with the third investigator (HHA/WST).

2.4. Quality assessment

Using the AMSTAR-2 (a critical appraisal tool for systematic reviews that include randomised or non-randomised studies-2) tool (Shea, et al., 2017), two investigators (CN, HN/HHA) independently graded the quality of each meta-analysis identified for this review. Any differences between the two assessors were settled by consensus. The quality of primary studies included in each meta-analysis were not evaluated.

2.5. Statistical analysis

From each meta-analysis identified, the summary mean estimates along with the 95% confidence interval (CI), number of included

primary studies, total sample sizes, heterogeneity level (i.e., I^2 statistics), and information about publication bias were collected if the results were reported with a random effects model. In case, it was done with fixed-effect model, we re-calculated the meta-analysis using the random-effects model to enhanced accuracy.

Summary effect: For pooling of meta-analysis in this umbrella review, standardized mean difference (SMD) (estimated by Hedges' g) and its 95%CI were calculated. If newly published primary studies were found after the publication of particular meta-analysis, the effect size of that meta-analysis was updated by adding data extracted from the new studies. This is the case for six published meta-analyses.

Heterogeneity between studies: For the summary random effects estimates, the 95% prediction interval (PI) was calculated. Considering heterogeneity under consideration, the PI approach provides a 95% certainty for the estimated range of true effects in comparable studies (Riley et al., 2011; IntHout et al., 2016).

Largest study: In order to accurately represent the study size, the largest study in individual meta-analysis was detected with the use of smallest standard error (SE), as described elsewhere (Ioannidis, 2013).

Excess significance: In order to assess whether the observed number of studies (O) with nominally significant results ("positive" studies, $p < 0.05$) differed from the expected number of significant results (E), the excess significance test was done for each single meta-analysis. The threshold for excess significance for nine single meta-analyses (i.e., meta-analysis for each nine cytokines in this case) was established at < 0.01 (i.e., one sided $p < 0.05$ with $O > E$ (Ioannidis and Trikalinos, 2007; Ioannidis, 2013).

Small study effects: Information about the small study effects and publication bias was obtained from the included me-analyses, if available. Otherwise, it was investigated with funnel plot asymmetry and Egger's test (if 10 or more primary studies were included) (Egger et al., 1997; Sterne et al., 2001).

For data analysis, *metafor* and *metaumbrella* package in *R*® (4.3.2), and *metan* package in STATA (version 16, TXT) were used.

2.6. Credibility assessment

As described elsewhere (Ioannidis and Patsopoulos, 2007; Ioannidis, 2009), variables such as the number of studies, the number of participants, and I^2 statistics were taken into consideration for the credibility assessment criteria. Four classes (Class I, convincing; Class II, highly suggestive; Class III, suggestive; and Class IV, weak) were then used to determine the evidence. More description about the classification is provided in Supplementary File 3.

3. RESULTS

The study selection procedure appears in Fig. 1. A database search produced the initial 135 studies. In the final analysis, seven meta-analyses (Hashmi, et al., 2023; Kotepui, et al., 2022, 2023a; Kotepui, et al., 2023b; Mahittikorn et al., 2022; Wilairatana et al., 2022a, 2022b) were identified and due to availability of primary studies, two

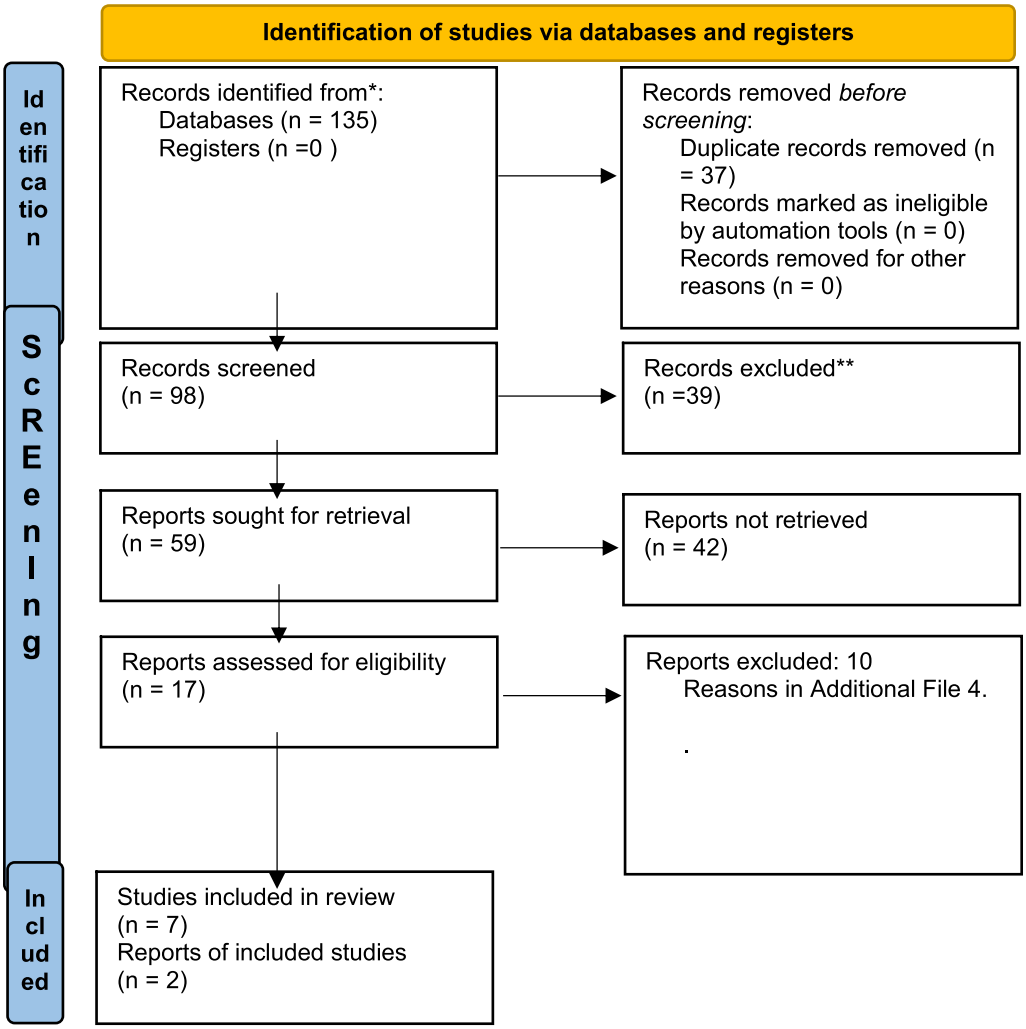

Fig. 1. Study selection process.

meta-analyses for IL-2 and IL-13 were added by this research team. Hence, this umbrella reviews included a total of nine meta-analyses (n = 12,674). The excluded meta-analyses were provided in Supplementary File 4.

3.1. Description of meta-analyses

Table 1 presents the main characteristics of the nine meta-analyses that reported nine unique cytokines (i.e., IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, and TNF-α). There was only one meta-analysis of observational studies on each of the nine cytokines for the outcome of malaria severity. The number of included primary studies in these nine meta–analyses spanned from three studies (for an IL-2 meta-analysis by the present research team) to 19 studies (for an IL-10 meta-analysis; Hashmi et al., 2023), with most frequent seven studies in the IL-Iβ, IL-4, or IL-8 meta-analyses (Mahittikorn et al., 2022; Kotepui et al., 2022, 2023b). Total number of participants in these nine single meta-analyses varied from 217 (for an IL-2 meta-analysis) to 2539 (for an IL-10 meta-analysis). Data from individual primary studies identified in nine single meta-analyses are provided in Additional File 5. Overall, the methodological quality of nine meta-analyse was 'high' due to absence of critical weakness or presence of only one non-critical weakness (Supplementary File 6).

3.2. Summary of effect sizes

Fig. 2 shows the effect estimates and their directions using equivalent SMD/ Hedge g for the relationship between cytokines and severe malaria**.** Based on the random effects model, only one meta-analysis for IL-1β of the nine meta-analyses (11%) showed significantly higher levels of cytokines in those with severe malaria than those with uncomplicated malaria (p: 0.009). The 95% PIs were not statistically significant in any one of the nine meta-analyses identified for this review (Table 1, Supplementary File 7).

3.2.1. Heterogeneity between studies

There was evidence of substantial heterogeneity (I^2 test: 73% to 99%) in all nine meta- analyses (Table 2).

3.2.2. Small study effects

Of nine, three individual meta-analysis of IL-6, IL-10 and TNF (3/9, 33%) had significant small study effects (Table 2).

3.2.3. Excess significance

Two individual meta-analysis (i.e., meta-analyses of IL-4 and IL-12) (2/9, 22%) reported an evidence of excess significance bias (Table 2).

3.3. Evidence criteria

Of all nine cytokines identified in this umbrella review, only IL-1β showed "Class III evidence" that this cytokine was "suggestive" of contributing to malaria severity compared with uncomplicated malaria. The remaining cytokines assessed showed Class IV evidence, reflecting "weak" evidence on the impact of malaria severity (Table 3).

4. Discussions

4.1. Principal findings

The current umbrella review provides an extensive review of evidence on the relationships between malaria severity and cytokine levels. The upregulation of immune responses, including apoptosis induction, macrophage activation and the recruitment of additional immune cells is involved in pro-inflammatory cytokines production and these cytokines are then involved in further upregulation of immune responses, Pro-inflammatory cytokine production is linked to the elevation of immune responses, which involves apoptosis induction, macrophage activation, and the recruitment of additional immune cells. These cytokines then contribute to additional immune response upregulation (Chen et al., 2023). On the other hand, anti- inflammatory cytokines secreted from immune cells, such as regulatory T cells and some macrophages, served to suppress inflammation and immunity (Dobb et al., 2020; Popa and Popa, 2021). The list of anti-inflammatory cytokinesis is large, but this umbrella review, based on the available data, focused on nine

Table 1
Characteristics of the included nine meta-analyses.

No. of meta-analysis.	Cytokines in meta-analysis	Number of primary studies[$]	Total/ SM	Summary ES			Fixed p	Rand p	95% PI	REmarks
				F	R	Largest				
1	IL1β	7(6)	1602/ 597	0.32 (0.2 to 0.43)	0.36 (0.11 to 0.62)	0.33 (0.1,0.56)	<0.001	**0.009**	−1.17, 1.9	Pf: 65%
2	IL2	3	217/ 115	0.34 (0.04 to 0.63)	0.54 (−0.18 to 1.26)	0.65 (0.19, 1.1)	0.025	0.16	−19.39, 20.46	Pf:100%
3	IL4	7	593/ 278	−0.41 (−0.59 to −0.23)	−0.82 (−1.87 to 0.23)	−0.77 (−1.26, −0.28)	<0.001	0.22	−7.43 to 5.79	Pf: 72.2%
4	IL6	14(13)	2206/ 786	0.49 0.38 0.6	0.93 (−0.02 to 1.88)	0.93 (0.46, 1.4)	<0.001	0.3	−4.8 to 6.66	Pf:74.4%
5	IL8	6(2)	1344/ 352	0.48 (0.34 to 0.63)	2.88 (0.83 to 4.93)	0.47 (0.02 to 0.92)	<0.001	0.18	−10.03 to 15.79	Pf:73.5%
6	IL10	21 (19)	2539/ 856	−0.49 (−0.59 to −0.39)	−0.5 (−1.19 to 0.19)	0.84 (0.42 to 1.25)	<0.001	0.06	−4.89 to 3.89	Pf: 68.4%
7	IL12	9(10)	1233/ 638	0.01 (−0.12 to 0.13)	−0.87 (−2.02 to 0.28)	0.38 (0.21 to 0.55)	<0.001	0.25	−7.7 to 6.03	Pf:80%
8	IL13	4	514/ 200	0.74 (0.47 to 1.02)	1.67 (- 3.53 to 6.86)	−2.69 (−3.63 to −1.76)	<0.001	0.53	−34.99 to 38.32	Pf: 88.7%
9	TNF-α	17(6)	2426/ 721	0.12 (0.01 to 0.24)	0.23 (−0.92 to 1.37)	0.59 (0.25 to 0.93)	<0.001	0.32	−6.76 to 7.21	Pf:61.5% (73.6%)**

Pf: *Plasmodium falciparum.*

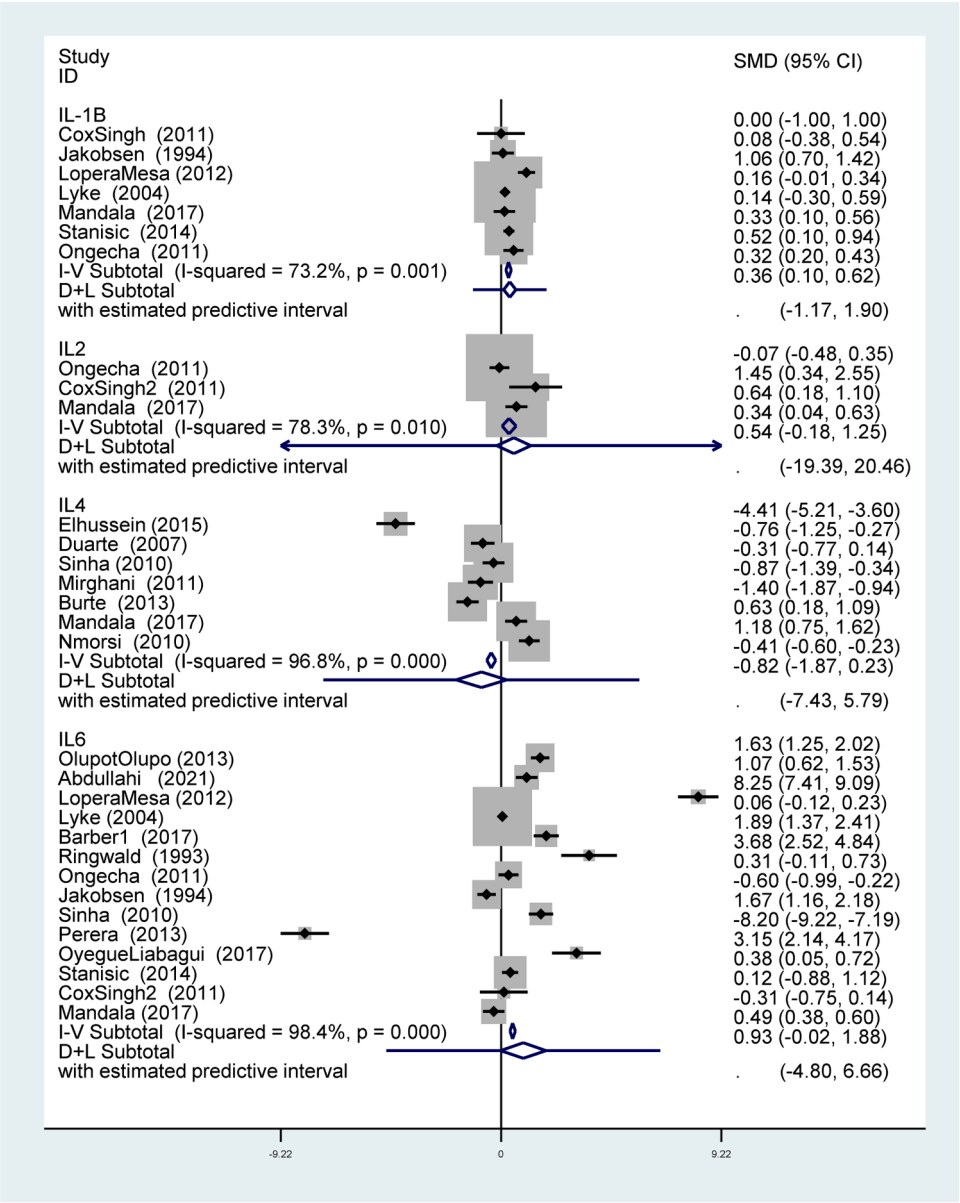

Fig. 2. Effect estimates showing the impact of IL-1β, IL-2, IL-4, and IL-6 cytokines levels in severity of malaria.
Note: A vertical line in the centre is the line of null value. A horizontal line representing each study. The width of the line represents the 95% interval. The diamond/point/square in the centre of the line is a point estimate of the true value. The bigger the shape, the larger the sample size. A diamond at the base of the graph represents a weighted average of effect estimate for all studies. SMD: standardized mean difference. For example, Mandala 2017 reported SMD 0.49 (95% CI: 0.38 to 0.6). I^2 value in% represents magnitude of between-study heterogeneity.

cytokines (i.e., IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, and TNF-α).

A pooled analysis of nine individual meta-analysis covered 12,674 participants and nine distinct cytokines (i.e., IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, and TNF-α). Higher levels of the pro-inflammatory cytokines were observed in severe malaria as compared to uncomplicated malaria across the pooled analyses. In the current analysis, IL-10 tended to be higher in those with severe malaria. A prospective study on Vietnamese adults reported that higher regulatory relationship of IL-6 to IL-10 ratio was significantly associated with severe malaria, and even deaths (Day et al., 1999).

Regarding evidence classification criteria assessments, only one of the nine meta-analyses (11%) provided "suggestive" evidence (class III), and the remaining eight meta-analyses were with "weak" evidence. The observed heterogeneity was substantial in all these nine meta-analyses, and this might be related to variation in individual primary studies involved in respective meta-analysis. As described in a published umbrella review, clinical heterogeneity may be substantial even in the absence of statistical heterogeneity (Li et al., 2021).

4.2. *Biological plausibility*

Cytokines are crucial for modulation of the immune responses in malaria (Popa and Popa, 2021). Higher levels of cytokines are linked to anaemia, impaired liver function, and fever on one side, and parasite control on another side. Thus, cytokines serve as key mediators in the pathogenesis of malaria (Dobb et al., 2020).

Fundamentally, certain cytokines exacerbate the disease by promoting inflammation (proinflammatory), while others aid in reducing inflammation and facilitating healing (anti-inflammatory) (Dinarello, 2000). The pro-inflammatory cytokines are secreted from Th1 cells, $CD4^+$ cells, macrophages, and dendritic cells (Souto et al., 2014).

Table 2
Evaluation of bias and heterogeneity in meta-analysis of cytokines for severity of malaria.

No.	Cytokine in meta- analysis	Number of studies	Egger's P value	I^2% (95% CI)#	Excess significance		
					Observed	Expected	P value of EST
1	IL-1β	7	NA	73.3 (43–81)	3	2.14	0.2404
2	IL-2	3	NA	79.4 (35–94)	2	1.1	0.1403
3	IL-4	7	NA	97 (96–98)	8	0.57	**< 0.0001**
4	IL-6	14	**<0.001**	98 (97–98.2)	10	12.3	0.939
5	IL-8	7	NA	99.1 (99–100)	2	2.61	0.693
6	IL-10	21	**<0.001**	91 (87–93)	9	7.95	0.318
7	IL-12	9	NA	98.3 (98–99)	9	0.48	**<0 0.0001**
8	IL-13	4	NA	99.4 (99–100)	2	1.27	0.215
9	TNF	17	**<0.001**	98.6 (98–99)	14	17.25	0.999

Bold indicates statistically significant at $p \leq 0.05$.

Table 3
Evidence classification.

No.	Meta-analysis of cytokine	Number of included studies	Evidence
1	IL-1β	7	Class III
2	IL-2	3	Class IV
3	IL-4	7	Class IV
4	IL-6	14	Class IV
5	IL-8	7	Class IV
6	IL-10	21	Class IV
7	IL-12	9	Class IV
8	IL-13	4	Class IV
9	TNF-α	17	Class IV

Pro-inflammatory IL-1β, IL-2, IL-12, and TNF-α were identified in the current umbrella review. Severe malaria has been associated with higher pro-inflammatory to regulatory cytokine ratios (Dodoo et al., 2002). TNF-α along with other pro-inflammatory cytokines play a crucial role in controlling the parasite growth and its elimination in malaria. For instance, TNF-α along with gamma IFN released when CD8+*T* cells are stimulated, shows an increased release of nitric oxide in the hepatocyte to kill the parasites. TNF-α also released from activated dendritic cells regulates innate and adaptive immunity. There is an increased phagocytic uptake of parasites as a result of the TNF-α effect. (Poppa and Poppa 2021).

The significant association of pro-inflammatory cytokines IL-1β, with severe malaria as observed in this umbrella review, has biological plausibility. IL-1β is a potent proinflammatory cytokine, primarily produced by lymphocytes, monocytes, and macrophages in response to microbial molecules. It activates $CD4^+$ cells and directs their differentiation towards Th17 cells (Ede, 2009).

IL-6 is one of the proinflammatory as well as a pleiotropic cytokine (Uciechowski et al., 2020) that can affect the immune system, contributing to an increased disease severity. Although exact mechanisms are not fully understood, this might be due to an increasing parasitaemia load (Oyegue-Liabagui et al., 2017). IL-6 was not significantly associated with severity of malaria in the current umbrella review, and this might be due to limited number of parasitaemia in participants of the primary studies. Regulatory cytokines such as IL-10 (in this case) act as a crucial role in maintaining the balance between the pro-and anti-inflammatory responses. When this equilibrium is disrupted, an exaggerated proinflammatory response occurs, resulting in significant adverse effects attributed to severe forms of malaria and subsequent deaths (Popa and Popa, 2021). The IL-10 levels in severe malaria may be related to parasitaemia. For instance, during the acute phase, IL-10 level was positively correlated with parasitaemia at $\rho = 0.62$, $p < 0.001$ (Luty et al., 2000). Patients with uncomplicated infection may produce more IL-10, thereby enhancing their capacity to restrain proinflammatory reaction. Thus, the pathogenesis of severe falciparum malaria might involve not only an excessive proinflammatory response but also a defective negative feedback mechanism (Ho et al., 1998). The correlation observed between pro-inflammatory cytokines and IL-10 implies that IL-10 cats as a regulator in preventing excessive production of pro-inflammatory cytokines (Wautier and Wautier., 2023).

A concern is that synergism may occur amongst certain cytokines such as IFN-γ and TNF-α, and this can lead to a significantly greater biological effect than what would be anticipated levels of individual cytokines (Day et al., 1999).

4.3. Study limitations

We acknowledge some limitations. First, the quantification of cytokine concentrations through immunoassays depends heavily on the specificities of the antibodies used and this can create the wide variations in the absolute values amongst different assays (Day et al., 1999). This might cause a likely bias in the meta-analysis if individual primary studies vary in the quantification of cytokine concentrations. Second, most cytokines undergo rapid clearance, with half-lives typically lasting only minutes. Consequently, circulating levels can be varied by the time of sampling and the duration of the stimulus for synthesis (Day et al., 1999). Additionally, use of various anticoagulants, tube contamination by endotoxins, and delays in blood processing (centrifugation) can have a major impact on cytokines concentrations in plasma or serum and result in falsely increased or decreased cytokine measurements (Liu et al., 2021). Hence, cytokine in the primary studies of the selected meta-analyses might not be at the actual levels. Last but not the least, if only published meta-analyses were included, there might have been primary studies that were not captured in these existing systematic reviews/meta-analyses. However, this was less likely because if found, eligible individual primary studies were added to the existing meta-analyses for comprehensive estimates in this umbrella review.

This review only provides a selected number of cytokines that are important for inflammatory reactions in human body. Although numerous other cytokines, hormones, and protein can mediate the immune dysregulation (Kany et al., 2019), there were insufficient systematic reviews for the other cytokines thereby excluding them from this umbrella review.

4.4. Implications

A successful host immune response is generally the outcome of both pro- and anti-inflammatory cytokines that are carefully orchestrated with the goal of pathogen clearance (malaria parasites in this case) and limiting host damage (Cicchese et al., 2018).

Dysregulation of the cytokine network in severe malaria is related to parasites and host factor variations. A review reported that cytokines involved in malaria (TNF-α, IL-4, and IL-10) play a double role as a friend or an enemy (Popa and Popa, 2021). On consideration of the short half-life of cytokines, proper sample preparation and procession is important to ensure that the analysis is accurate and reliable (Liu et al., 2021).

Clinical practice has been shown to benefit from the identification and validation of biomarkers for severe malaria, particularly in low-income settings with a shortage of trained clinical personnel and diagnostic resources. However, Foko et al. (2022) pointed out that small sample sizes, the quality of the studies limited the strength of the evidence, and specificity for malaria often limit the validation of various potential candidates, as also observed in this umbrella review.

It appears that a unique single biomarker would not be reliable for predicting malaria severity in endemic and non-endemic settings. Based on the cytokine activation pathway and their correlations, a combination of these biomarkers would be effective by improving the specificity and representing a reliable early diagnostic method (Monastero and Pentyala, 2017; Hashmi et al., 2023).

There are unmet needs to develop a technology which can conduct measurements in real-time with high accuracy and efficiency, and to recognize and differentiate between numerous cytokines concurrently while providing point of care output signal efficiently and swiftly (Liu et al., 2021).

5. Conclusion

Findings suggest that, compared to uncomplicated malaria, the pro-inflammatory cytokine IL-1β contributes to the development of severe falciparum malaria. Due to the limited level of evidence, further well-designed larger studies with multiple cytokines are needed to investigate the use of cytokine levels as reliable biomarkers in malaria severity.

Consent for publication

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CRediT authorship contribution statement

Cho Naing: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Han Ni:** Methodology, Investigation, Formal analysis. **Arun Kumar Basavaraj:** Investigation, Formal analysis, Data curation. **Htar Htar Aung:** Methodology, Investigation, Data curation. **Wong Siew Tung:** Methodology, Investigation. **Maxine A Whittaker:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2024.107447.

Data availability

All data are available in the manuscript and supplementary files. Thank you

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