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Review

Markers of dengue severity: a systematic review of cytokines and chemokines

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The prognosis of dengue remains a challenge in the early, objective triage of patients with dengue fever of differing severity. Circulating immuno-modulating proteins have brought new possibilities as prognostic markers of severe dengue (SD). This systematic review is devoted to understanding the potential utility of blood-based cytokines and chemokines as prognostication markers of SD based on the current literature. PubMed and Embase were searched. Of 794 candidate articles, 685 abstracts were screened against our exclusion/inclusion criteria and 25 (3.6 %) studies met the quality assessments. A total of 18 studies were retrospective observational and 2 were prospective cohort studies. Elevated IL-10, up to day 7 of fever onset, stood out as a candidate prognostic marker for SD using the 1997 and 2009 World Health Organization (WHO) case definitions. IFNγ was another potential prognostic marker of SD (1997 WHO case definition), but its levels varied between studies. Significant heterogeneity in methodologies and patient cohorts prevent ready application of IL-10 and IFNγ as prognostic markers to other dengue populations. Our results suggest that the current non-randomized studies are delivering inconsistent messages and higher-quality studies, with consistent methodologies and validation in independent patient cohorts, are needed to delineate confounding variables. Major gaps identified were full accounting and transparency of sampling days, dengue virus type, infection status and age group.

Introduction

Dengue is an emerging arboviral threat globally as its spread and incidence is moving on an upward trajectory, infecting some 390 million people yearly (Bhatt *et al.*, 2013). Dengue can be asymptomatic, or present as a self-limiting dengue fever (DF), and in certain patient subsets the disease may precipitate into the severe, potentially life-threatening forms of dengue – dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) or severe dengue (SD). In the 1997 World Health Organization (WHO) case definition, patients were diagnosed as having DHF if they had fever, thrombocytopenia, bleeding and evidence of plasma leakage (hypoproteinaemia, change in haematocrit of more than 20 % or clinical fluid accumulation) (WHO, 1997). DSS has all the features of DHF plus circulatory failure in the form of rapid and weak pulse, and narrow pulse pressure (<20 mm Hg), or age-specific hypotension and cold, clammy skin and restlessness. In the 2009 WHO case definition, patients were diagnosed as SD if they had severe plasma leakage, severe bleeding and severe organ involvement (aspartate aminotransferase or alanine aminotransferase$\geq$1000 U/L, impaired consciousness, or failure of heart and other organs) (WHO, 2009). Dengue can be caused by any of the four dengue types of the genus *Flavivirus*. The propensity to develop SD is plausibly brought about by a number of factors, including heterologous secondary dengue infection, dengue type, age or a combination of these (Kyle & Harris, 2008; OhAinle *et al.*, 2011). There are no effective antiviral therapeutics to treat dengue and the current most advanced vaccine has an imbalanced efficacy against the four dengue types (Capeding *et al.*, 2014; Wilder-Smith & Massad, 2016). In view of this, early prognosis of SD, preferably less than 96 h from onset of fever, can guide patient triage, allow informed clinical decisions, and reduce disease morbidity and mortality.

The dynamic nature of dengue presents significant challenges in clinical management and on health services,

Three supplementary tables are available with the online Supplementary Material.

especially during an outbreak. Currently, routine haematological and biochemical measurements in dengue patients collectively correlate poorly with eventual clinical outcome, there are no tests to differentiate those who will have DF from those who will progress to and deteriorate to SD. In the absence of any effective treatment against the disease, proper fluid management is critical. Guidelines produced by the WHO list a number of warning signs to help inform clinical decision making, but they all have poor specificity resulting in large numbers being admitted unnecessarily (Thein *et al.*, 2013). Over-hospitalization of dengue patients is contributed to in part by the inability to prognosticate early in the febrile phase, and resulted in excessive hospitalization rates and costs (Lee *et al.*, 2013). The unpredictable nature of outbreaks often overwhelms already fragile health-care systems. Prognosis is also important for monitoring dengue patients who display probable warning signs of health deterioration or complications that warrant further investigation. A prognostic marker is defined as a clinical or biological characteristic that is objectively measurable and that provides information on the likely outcome of the disease in an untreated individual (Hayes *et al.*, 1998).

Faced with the limited utility of current methods of SD prognosis, there has been interest in investigating the utility of systemic soluble factors as potential markers of SD prognosis. The two main prevailing hypotheses of DHF/DSS pathogenesis, antibody-dependent enhancement (ADE) and original antigenic sin (or T cell immunopathology), centre on an imbalanced immune system during secondary dengue infection (Halstead & O'Rourke, 1977; Mongkolsapaya *et al.*, 2003) triggering an exaggerated and imbalanced inflammatory cascade (Srikiatkhachorn & Green, 2010). In addition to secondary infections, the waning maternally-derived anti-dengue virus (DENV) IgG antibodies and/or an altered cytokine profile may explain SD in infants with primary infections (Libraty *et al.*, 2009; Nguyen *et al.*, 2004). Soluble factors emanating from immune cells (T cells, B cells, macrophages, mast cells, dendritic cells, etc.), platelets, stromal and endothelial cells in the form of cytokines and chemokines act as signalling molecules synergizing with one another to orchestrate cell growth and proliferation, differentiation/maturation, and immunity; hence, modulating host responses to infections (Fink *et al.*, 2006). Cytokines and chemokines are small proteins typically ranging from 8 to 40 kDa, and in this review we broadly refer to them as *immuno-modulating proteins*. Cytokines are secreted proteins that play a role in cell signalling, in the induction, inhibition and effector phases of immune and inflammatory responses. Chemokines are a subset of small cytokines that recruit and exert chemotactic migration of other cells to a localized area to exert a variety of biological effects, including inflammation and homeostasis. Immuno-modulating protein profiles change with the clinical course of dengue, differ between DF and SD (Kumar *et al.*, 2012; Rathakrishnan *et al.*, 2012), and are believed to have direct impact on the manifestations of increased vascular permeability, plasma leakage and thrombocytopenia (Green & Rothman, 2006; Murphy & Whitehead, 2011). Immuno-modulating proteins have been proposed to cause a shift from the predominant TH_1-type response in DF to the TH_2-type in severe DHF (Fink *et al.*, 2006); because molecular signalling seemingly precedes gross morphological or observable clinical symptoms, the potential use of immuno-modulating proteins as early prognostic markers is especially welcoming (Lee & Ooi, 2013). However, studies in humans have resulted in varied responses and remained conflicting, with no objective consolidation of data having taken place so far. Hence, this systematic review aims to be a collection and summary of primary research articles that focuses on the ostensible utility of soluble immuno-modulating markers for the prognosis of SD.

Results

Study selection

We ran searches on PubMed and Embase, and a total of 794 references were retrieved: PubMed (n=451) and Embase (n=343). After excluding 109 duplicates, 685 records were screened on the basis of title and abstract against our exclusion/inclusion criteria, thereby identifying 54 potentially eligible records (Table S1, available in the online Supplementary Material). Two studies were excluded because the main text could not be retrieved or had incomplete methods. Another 30 records were excluded (4 low quality, 25 medium quality and 1with ambiguous sampling time). Some of the studies were further moderated based on empirical evidence leading to six studies being downgraded and one study being upgraded (Table S1). A total of 24 studies were identified as eventual eligible articles of high quality. The study selection flow chart is shown in Fig. 1. We restricted the search to 'English' language and 'Human' subjects. Three studies were excluded despite good study design due to a lack of comparison to DHF/DSS or SD (medium to high quality). Detection bias (blinding of outcome assessors) was relatively homogeneous across these non-randomized studies (NRS) and leaves all studies at approximately the same level of risk of bias and, thus, not considered.

Study characteristics

A total of 19 out of 24 studies were case–control studies (Arias *et al.*, 2014; Bozza *et al.*, 2008; Brasier *et al.*, 2012; Butthep *et al.*, 2012; Chen *et al.*, 2005; Furuta *et al.*, 2012; Green *et al.*, 1999a, b; Guerrero *et al.*, 2013; Houghton-Triviño *et al.*, 2010; De La Cruz Hernández *et al.*, 2014; Laur *et al.*, 1998; Levy *et al.*, 2010; Malavige *et al.*, 2013a; Nguyen *et al.*, 2004; Del Moral-Hernández *et al.*, 2014; Pérez *et al.*, 2004; Soundravally *et al.*, 2014; Wang *et al.*, 2007), and the remaining 5 studies were prospective cohort studies (de-Oliveira-Pinto *et al.*, 2012; Kumar *et al.*, 2012; Kurane *et al.*, 1991, 1993; Suharti *et al.*, 2003). Comparison groups were healthy controls, patients with other febrile illness (OFI) or non-severe disease patients (DF or non-SD); however, comparisons made between SD and non-SD cases were considered most ideal for the purpose of this review. Study characteristics of the

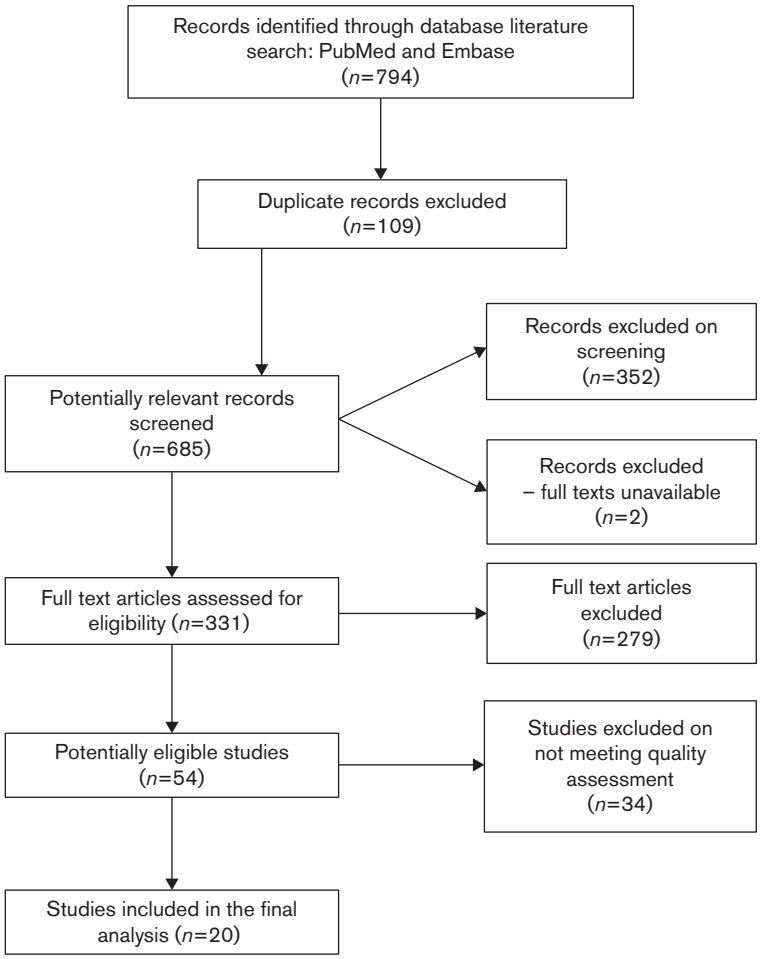

Fig. 1. Flow diagram of the search and review process.

24 studies are reported in Table 1. One study did not specify which WHO case definition was applied, but this was verified with the authors and the manuscript was subsequently accepted for further evaluation (de-Oliveira-Pinto *et al.*, 2012).

Ten studies noted affirmatively that their first blood sampling was performed at the febrile phase, which we defined here as <5 days or <96 h from fever onset (Brasier *et al.*, 2012; Butthep *et al.*, 2012; Green *et al.*, 1999a, b; Kumar *et al.*, 2012; De La Cruz Hernández *et al.*, 2014; Malavige *et al.*, 2013a; Pérez *et al.*, 2004; Soundravally *et al.*, 2014; Wang *et al.*, 2007). The remaining studies had wider blood sampling windows, ranging from 1 to 10 days from symptom onset (Arias *et al.*, 2014; Bozza *et al.*, 2008; Chen *et al.*, 2005; de-Oliveira-Pinto *et al.*, 2012; Guerrero *et al.*, 2013; Houghton-Triviño *et al.*, 2010; Kurane *et al.*, 1991, 1993; Laur *et al.*, 1998; Levy *et al.*, 2010; Del Moral-Hernández *et al.*, 2014; Nguyen *et al.*, 2004; Suharti *et al.*, 2003). A majority of studies (21/24, 87.5 %) employed ELISAs for quantification of single cytokine/chemokine, or multiplexed suspension bead immunoassays for broader profiling. Three studies (12.5 %) used cytometric bead arrays (Guerrero *et al.*, 2013; Houghton-Triviño *et al.*, 2010; De La Cruz Hernández *et al.*, 2014).

Assessment of study design eligibility and risk of bias in individual studies

Table S2 summarizes the risk of study design eligibility and bias of selected studies according to the Cochrane NRS Methods group. First, all studies compared DHF, DSS or SD with DF, dengue without warning signs (DwoWS) or dengue with warning signs (DwWS) groups, as this was part of our quality assessment criterion (Table 2). Therefore, risk of bias in this item was negative. We considered the risk related to retrospective design as moderate, because the outcome assessments were retrospective and the generation of the hypothesis was prospective. Detection risk was considered low because patients were categorized according to canonical and accepted definitions of dengue severity clinical symptoms (WHO, 1997, 2009) and not by patients' preference or marker (assay) outcomes. One study applied modified WHO guidelines (Bozza *et al.*, 2008). However,

Table 1. Characteristics of included studies

No.	Author and publication year	Study design	Sample size (*N*)	Age	Measured immuno-modulator	Sampling time and follow-up (where applicable)	Ascertainment of dengue diagnosis	Endpoint measured
1	Bozza *et al.* (2008)	CC	DF=20, DHF=39; total=59	DF, 28–48 years; DHF, 23–53 years	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, CXCL8 (IL-8), IL-10, IL-12 (p70), IL-13, IL-17, GM-CSF, MCP-1, MIP-1β, TNFα	3 and 10 days after disease onset	WHO (1997)*	(1) Increased IL-1β, IFNγ, IL-4, IL-7, IL-13, GM-CSF in DHF compared to DF; (2) increased MIP-1β levels in DF compared to DHF; (3) MIP-1β and IFNγ were independent variables associated with disease outcome – MIP-1β increased during mild dengue with OR=0.181, while IFNγ was associated with severity with OR=1.138
2	Brasier *et al.* (2012)	CC	DF=38, DHF=13; total=51	DF, 15.8±7.8 years; DHF, 19 ±13.4 years	IL-2, IL-6, IL-10, IFNγ, IP-10, MIP-1α, TNFα, TRAIL, VEGF	Day 1 upon fever onset	WHO (1997)	(1) Increased log2-IL-10 and log2-IL-6 in DHF compared to DF; (2) increased IL-10 concentration associated with increased probability of DHF (using logistic regression model)
3	Butthep *et al.* (2012)	CC	OFI=15, DF=51, DHF=98; total=164	Not stated	EGF, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFNγ, MCP-1, TNFα, VEGF	−3 days from defervescence	WHO (1997)	(1) Increased IL-4 in DHF II and DSS compared with DF, DHFI and OFI only at day −1; (2) increased levels of IL-6 and IL-8 in DSS than in DF, DHFI, DHFII and OFI on day +1 and day +2 compared with the other groups at day −2 to +2; (3) IFNγ and IL-10 – highest level was detected in DSS compared with DF, DHFI and DHFII on day −1, whereas the lowest level was detected in OFI; (4) increased TNFα in DF, DHFI, DHFII and DSS than in OFI from day −2 to 0 except for DSS on day 0.; (5) increased IL-1β and IL-2 in DF than in DHF and OFI from day −1 to +2, the lowest IL-2 was found in DSS except on day +2
4	Chen *et al.* (2005)	CC	DF=66, DHF=33 (mild DHF=24/33, severe DHF=9/33); total=99	DF, 20–81 years; DHF, 30–76 years	IFNα, IFNγ, IL-10, IL-13	1–7 days upon symptom onset	WHO (1997)	(1) Increased IFNγ in DF compared to DHF (P=0.01); (2) increased IL-10 in DHF compared to DF (P=0.03); (3) no difference in IFNα and IL-13 between DF and DHF; (4) significant correlation between disease severity and IL-10 (P<0.001) but not IFNα, IFNγ or IL-13
5	De La Cruz Hernández *et al.* (2014)	CC	DF=116, DHF=88; total=204	Not stated	IFNα	First 5 days post-fever onset	WHO (1997)	Increased IFNα levels in DF than DHF for both DENV1 (P=0.0052) and DENV2 (P=0.0233)
6	Del Moral-Hernández *et al.* (2014)	CC	Controls=81, DF=70, DHF=80; total=231	0–95 years	sTM, VEGF	1–10 days post-fever onset	WHO (1997)	(1) Increased sTM level in DHF> DF (P<0.001), DF> controls and DHF>controls (P<0.0001); (2) increased VEGF in DF compared to DHF (P=0.005), DF compared to control and DHF compared to control (P=0.0001); (3) increased sTM levels in DENV2 compared to DENV1, no significant difference for VEGF with serotype
7	Furuta *et al.* (2012)	CC	DF=19, DHF=43, DSS=41; total=103	6 months–15 years	IL-9, IL-17m VEGF (VEGF-A), sVEGFR-1, sVEGFR-2	Blood samples collected at time of admission (day 0) and twice during the following 4 days (day 2 and 4)	WHO (1997)	(1) Increased VEGF in DHF and DSS than DF and controls (P<0.01) at day 0; (2) increased sVEGFR1 in DSS than DHF, DF and control (P<0.01); (3) decreased sVEGFR2 in DHF and DSS compared to DF and control (P<0.01);

Table 1. cont.

No.	Author and publication year	Study design	Sample size (*N*)	Age	Measured immuno-modulator	Sampling time and follow-up (where applicable)	Ascertainment of dengue diagnosis	Endpoint measured
								(4) increased IL-9 and IL-17 levels in DHF and DSS compared to DF and controls at day 0
8	Green *et al.* (1999a)	CC	OFI=112, DF=32, DHF=28; total=172	6.8 years	IL-1β, TNF-α, IL-6, IL-4, IFN-γ, sIL2R, sCD8, sCD4, sTNFRI, sTNFRII	<72 h, equivalent to −2 days before defervescence	WHO (1997)	(1) Increased mean plasma sTNFRI levels in DHF than DF from −2 days before defervescence (P<0.01); (2) increased sIL2R and sCD8 levels in DHF compared with DF+1 after defervescence (P<0.001 and <0.05, respectively)
9	Green *et al.* (1999b)	CC	OFI=112, DF=32, DHF=28; total=172	6.8 years	IL-10, IL-12 p70, IL-12 (p40 +p70)	<72 h, equivalent to −2 days before defervescence	WHO (1997)	(1) Higher mean plasma IL-10 in DHF children compared to DF as early as 2 days before defervescence (P<0.05); (2) higher mean plasma IL-12 (p40+p70) DHF compared to DF from fever day −2 (P<0.05)
10	Houghton-Triviño *et al.* (2010)	CC	DF=21, DHF I/II=4, DHF III/IV=13; total=38	0.3–55 years	IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12, IFNγ, sST2, TNFα	2–7 days of the disease	WHO (1997)	Increased TNFα, sST2, IL-8, IL-10 and IL-4 DHF compared to DF (P<0.05)
11	Kumar *et al.* (2012)	PC	DF=44, DHF=18; total=62	DF, 39 years; DHF, 40 years	IL-1Rα, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-17, Eotaxin, FGF-basic, G-CSF, IFNγ, IP-10, MCP-1, MIP-1β, PDGF-BB, TNFα, VEGF	<72 h upon onset of fever	WHO (1997)	(1) Maximally increased in dengue patients during febrile phase – IFNγ, IP-10, IL-4, IL-10, IL-13, IL-1β, IL-6 and IL-8; (2) increased IP-10, IL-4, IL-9, IL-10, IL-1ra in dengue patients compared to controls during febrile phase; (3) decreased IFNγ, IL-4, IL-17 in DHF compared to DF during febrile stage
12	Kurane *et al.* (1991)	PC	Afebrile controls=97, DF=10, DHF=59; total=166	6–13 years (97 healthy children)	IL-2, IFNγ, sIL-2R, sCD4, sCD8.	Specimens collected within 24 h of admission to hospital and daily until discharge	WHO (1986); note – hospitalized cases of dengue that did not meet DHF criteria were classified as DF	(1) Increased sIL- 2R (P<0.05), sCD4 (P<0.001) and sCD8 (P<0.001) in DHF than DF; (2) IFNγ and IL-2 levels were not different between DHF and DF
13	Kurane *et al.* (1993)	PC	Controls=30, DF=10, DHF=35	Case, 5–14 years; control, 6–11 years; mean age (years) – DHF=9.1, DF=9.9, controls=7.9	IFNα	(1) Specimens collected within 24 h of admission to hospital and daily until discharge; (2) a convalescent specimen was collected from each child 7–10 days after hospital admission	WHO (1986)	(1) Increased IFNα in DHF than in controls on days 2–4, days 6–7 and days 10–20; (2) increased IFNα in DF than in controls on day 1 and day 3, but levels were not high on day 4–20 after onset of fever; (3) mean levels of IFNα in DHF patients were highest 2 days before deferevescence, and decreased gradually until day of defervescence, IFNα levels did not change during day 0–19; (4) IFNα levels in DF patients were high 1 day before and on the day of defervescence, but these levels were not high after fever subsided; (5) IFNα level did not differ among different groups with different DHF grades
14	Laur *et al.* (1998)	CC	DF=106, DHF=17;	1 month to 15 years (mean 85.9	TNFα, TGFβ−1	Days 1 to 8 post-fever onset	WHO (1997)	(1) Increased TGFβ−1 in DHF than in DF; (2) TNFα levels did not differ significantly between children with

Table 1. cont.

No.	Author and publication year	Study design	Sample size (*N*)	Age	Measured immuno-modulator	Sampling time and follow-up (where applicable)	Ascertainment of dengue diagnosis	Endpoint measured
			total=123	months, median of 88 months (not differentiated among DF and DHF)				DF and DHF
15	Levy *et al.* (2010)	CC	DF=36, DHF=34; total=70	3–53 years (not differentiated among DF and DHF)	IL-1β, IL-6, TNFα	1 to 6 days post-fever onset	WHO (1997)	(1) Increased IL-6 in DHF than DF (P<0.001); (2) increased TNFα than DF and DHF-I primary infection (P<0.01)
16	Nyugen *et al.* (2004)	CC	DHF=85, DSS=22; total=107	1–11 months (mean age of DSS was significantly higher than DHF)	IL-2, IL-4, IL-6, IL-10, IFNγ, TNFα	3–7 days post-fever onset	WHO (1997)	(1) Increased IFNγ from infants with DHF/DSS than healthy controls; (2) increased IFNγ in DHF/DSS on days 4–6 after onset of fever and rapidly decreased on day 7 during convalescence; (3) increased TNFα in infants with DHF/DSS in acute phase compared to controls; (4) increased TNFα on days 4–7 after onset of fever and decreased on days 8–19; (5) increased IL-10 and IL-6 in DHF/DSS infants compared to controls during acute phase
17	Pérez *et al.* (2004)	CC	Controls=12, DF=28, DHF=6; total=46	16–59 years old (not differentiated among controls, DF and DHF)	IL-10, IL-12 (p70+p40), RANTES	Every 48 h – 1st (between first and third days post-fever onset), 2nd (fourth or fifth day) and 3rd (after sixth day of clinical evolution)	WHO (1997)	(1) Increased IL-10 in DHF than DF (P<0.03) in 1st and 2nd determination; (2) lower RANTES in DHF than DF in 2nd and 3rd determination
18	Soundravally *et al.* (2014)	CC	DF=27, DHF=30, DSS=24; total=81	DF=22 (16–67) years; DHF=35 (20–59) years; DSS=24 (16–65) years	TNFα, IFNγ	4 or 5 days post-fever onset	WHO (1997)	(1) Decreased TNFα in DF vs DSS (P<0.001), increased IFNγ in DF vs DHF (P<0.001), increased IFNγ in DF vs DSS (P=0.028); (2) decreased TNFα in DHF vs DSS (P<0.001); (3) decreased TNFα/IFNγ ratio in DF vs DHF (P=0.039); (4) decreased TNFα/IFNγ ratio in DF vs DSS (P<0.001)
19	Suharti *et al.* (2003)	PC	DHF III=43, DHF IV=7; total=50	6.6±2.8 (3–13) years	TNFα, IL-1β, IL-1Rα, IFNγ, IL-6	4.2±1 (2–7) days in the acute phase of the disease	WHO (1997)	(1) Increased IL-1Rα in non-survivor DSS (802.2±566.4 vs 1566.9 ±675.6, P=0.0005); (2) IL-1Rα was significantly associated with mortality, P=0.007; (3) increased IL-6 in non-survivor DSS compared to survivors (219.50±58 262.0 vs 172.1±956.8, $P\leq$0.00001)
20	Wang *et al.* (2007)	CC	DF=44, DHF I/II=20, DHF III/IV=5; total=69	DF 50.2±2.7 years; DHF, 48.54±3.6 years	TNFα, sTNFRI and sTNFRII	DF, 4.09±0.32 days; DHF, 4.91±0.44 days post-fever onset	WHO (1997)	(1) Increased plasma sTNFRI in DHF than DF (5626±1270 vs 2846±212 pg ml^{-1}; P<0.04); (2) increased TNFα levels in DHF than DF (36.57±19.47 vs 349 ±0.36 pg ml^{-1}) (p=NS)
21	Arias *et al.* (2014)	CC	DwoWS=12, DwWS=10, SD=8, non-dengue	Control, 18 (2–42 years); DwoWS, 20 (5–33 years); DwWS, 12 (1–	IL-1β, IL-6, IL-12, IL-17, sTRAIL, sST2, sTNFRI, sTNFRII, TNFα	(1) Acute phase, 1–6 days after onset of symptoms; (2) convalescent, 7–26	WHO (2009)	(1) IL-6, IL-17, sTNFRI, sTNFRII, sST2 – control > DwoWS > DwWS > SD; (2) increased IL-12 and sTRAIL in DwoWS compared to control, DwWS and SD (P<0.05);

Table 1. cont.

No.	Author and publication year	Study design	Sample size (*N*)	Age	Measured immuno-modulator	Sampling time and follow-up (where applicable)	Ascertainment of dengue diagnosis	Endpoint measured
			controls=10; total=40	39 years); SD, 10 (4–26 years)		days after onset of symptoms; (3) recovery phase		(3) increased TNFα and IL-17 in SD compared to controls but no difference between different grades of disease severity
22	de-Oliveira-Pinto *et al.* (2012)	PC	Controls=16, DF=33, WS/severe=40; total=89; convalescent samples (n=26) were not taken into account	DF mean age, 37.33 years; WS/severe mean age, 38.2 years; controls mean age, 31.9 years	CCL2/MCP-1, CCL4/MIP-1β, CCL5/RANTES	2–9 days after first symptoms	Hybrid WHO (1997) and WHO (2009)	(1) Increased CCL2/MCP-1 in DF than WS/severe (P=0.002); (2) increased CCL4/MIP-1β in DF patients than WS/severe (P=0.04)
23	Guerrero *et al.* (2013)	CC	Controls=23, DwoWS=17, DwWS=21, SD=28; total=89	6–144 months (6 months – 12 years)	IL-1β, IL-6, IL-8, IL-10, IL-12p70, IL-33, sST2, TNFα	3–6 days post-fever onset	WHO (2009)	(1) Increased sST2 in SD than DwoWS and DwWS (P<0.001); (2) increased IL-6 and IL-8 in children with DwWS or SD compared to children with DwoWS (P<0.01); (3) increased IL-10 in DwoWS, DwWS, SD than controls (P<0.01)
24	Malavige *et al.* (2013a)	CC	Controls=15, non-SD=219, SD=40; total=274	Mean age (26.8 years) – SD=4.8, non-SD=5.25 (not differentiated among controls, non-SD and SD)	IL-10, IL-21, MIF	(1) Initial blood sample was obtained during day 4 and 5 of illness (day 1 was considered as first day of fever); (2) second blood sample was obtained in 65/259 patients during critical phase (2 days after obtaining the first blood sample)	WHO (2009)	Increased serum IL-10 in SD than non-SD

CC, case–control study; OR, odds ratio; PC, prospective cohort; WS, warning sign.
*Modified WHO (1997) guidelines.

we consider the confounding risk of prognostic factors moderate to high, because the type of immune-modulator under study was not uniform across the studies. A Kappa index of 0.91 was derived between two reviewers (W. Y. L. and Y. H. L.) suggesting substantial agreement.

Assessment of study design eligibility and risk of bias across studies

In this systematic review, which summarized mostly observational NRS, bias due to outcome differences may have arisen but we considered it as low to moderate as the usage and application of WHO case definition guidelines were enforced, and any potential differing opinions of disease severity minimized. However, comparability between studies utilizing the 1986/1997 or 2009 WHO revised case classification over time may be affected since the overlap between old and new case definitions are substantially different and as one or the other gains acceptance.

Prognostication marker candidates: WHO (1997) DHF I–IV

WHO (1986)/WHO (1997) was the most commonly applied case definition in the studies (20/24, 83 %) (Table 1). Studies differed in the types of immuno-modulating proteins investigated, and the most frequently profiled cytokines and chemokines were TNFα (12/20, 60 %), IFNγ (11/20, 55 %), IL-6 (10/20, 50 %), IL-10 (9/20, 45 %), IL-4 (6/20, 30 %) and IFNα (3/20, 15 %). Despite being the most investigated cytokine, TNFα was significantly elevated in DHF patients in only five studies (Houghton-Triviño *et al.*, 2010; Levy *et al.*, 2010; Nguyen *et al.*, 2004; Soundravally *et al.*, 2014; Wang *et al.*, 2007), and one study found no statistical difference in TNFα levels between DF and DHF patients (Laur *et al.*, 1998). The rest of studies reported no statistical difference or did not detect TNFα. By contrast, IL-10 stood out as the cytokine for which concentration was elevated in DHF relative to DF patients consistently in seven studies utilizing the WHO (1986)/WHO (1997) case definition (Table 3) (Brasier *et al.*, 2012; Butthep *et al.*, 2012; Chen *et al.*, 2005; Green *et al.*, 1999b; Houghton-Triviño *et al.*, 2010; Nguyen *et al.*, 2004; Pérez *et al.*, 2004). The association of dengue severity with circulating levels of IFNγ was more heterogeneous (Table 4) (Bozza *et al.*, 2008; Butthep *et al.*, 2012; Chen *et al.*, 2005; Kumar *et al.*, 2012; Nguyen *et al.*, 2004; Soundravally *et al.*, 2014). Three studies noted increased IFNγ in DHF and/or DSS patients relative to DF patients or healthy controls (Bozza *et al.*, 2008; Butthep *et al.*, 2012; Nguyen *et al.*, 2004), whereas three studies noted decreased IFNγ levels in DHF/DSS over DF patients (Chen *et al.*, 2005; Kumar *et al.*, 2012; Soundravally *et al.*, 2014). Bozza *et al.* (2008) noted defervescence phase IFNγ levels in adults (age 15–73 years) were associated with disease severity (odds ratio=1.138, 95 %confidence interval1.041 to 1.245, P=0.0046). However, Green *et al.* (1999a) reported no significant difference in febrile IFNγ between DF and DHF paediatric patients. In congruence, in infants (age <1 year), IFNγ (together with IL-2, IL-4, IL-6, IL-10, TNFα) could not differentiate DHF I/II from DHF III/IV (Nguyen *et al.*, 2004).

Prognostication marker candidates: WHO (1997) DHF III/IV

One study looked specifically at immuno-modulatory markers of predicting DSS (DHF III/IV) mortality (Suharti *et al.*, 2003). The study population was restricted to only DENV3-infected paediatric patients who were diagnosed as DSS in a prospective study. In the acute phase of dengue infection, plasma IL-1Rα levels were significantly higher in DSS non-survivors (n=7) compared to DSS survivors (n=43; 802.2±566.4 vs 1566.9±675.6 pg ml^{-1}, P=0.0005). Multiple logistic regression identified IL-1Rα as having significant association with mortality on the day of admission (P=0.007).

Prognostication marker candidates: WHO (2009) SD

Of the selected 24 studies, 4 studies (16.7 %) employed the WHO (2009) case definition (Arias *et al.*, 2014; de-Oliveira-Pinto *et al.*, 2012; Guerrero *et al.*, 2013; Malavige *et al.*, 2013a). Among them, one reported elevation in IL-10 (Malavige *et al.*, 2013a), congruent with the seven WHO (1997) guideline-utilizing studies that reported elevations in IL-10 in DHF patients (Table 3), and one study reported elevated IL-10 levels in DwoWS, DwWS and SD patients compared to controls (Guerrero *et al.*, 2013). Two studies reported elevations in sST2 (IL-1RL1) (Arias *et al.*, 2014; Guerrero *et al.*, 2013) in SD patients in levels above those of DwoWS and DwWS (Arias *et al.*, 2014; Guerrero *et al.*, 2013). The same studies reported increased IL-6 levels in SD and DwWS relative to patients with DwoWS. Elevations in TNFRI and TNRFII in DwoWS, DwWS and SD (Arias *et al.*, 2014) were similar to those observed in DHF patients (Wang *et al.*, 2007).

Discussion

Our systematic review of studies published from 1998 to 2014 evaluated cytokines and chemokines as potential early markers to prognosticate the development of SD. We evaluated and documented judgements about evidence quality from NRS and incorporated this into reporting of differentially changed cytokines and/or chemokines for outcomes based on WHO (1986)/WHO (1997) or WHO (2009) dengue case definitions. Twenty-four studies were identified to be of suitably high quality, but with major gaps in descriptions of patient demographics, DENV types and infection status (primary, secondary or tertiary infections). Although a consensus has yet to emerge, IL-10 levels stood out as a potential marker of SD (DHF and SD) that was significantly increased regardless of age (range 1 month to 76 years) or infection status (primary or secondary).

Eight studies utilizing WHO (1986)/WHO (1997) or WHO (2009) case definitions demonstrated elevated IL-10 levels in SD patients. Interestingly, several studies, including a

recent publication, showed similar IL-10 levels between patients with and without SD (Bozza *et al.*, 2008; Cui *et al.*, 2016; Guerrero *et al.*, 2013; Kumar *et al.*, 2012). The reason for this discrepancy is not entirely clear. IL-10 is an important anti-inflammatory cytokine and general suppressor of immune reactions, inhibiting IL-1, IL-6, IL-10 itself, IL-12, IL-18, CSF and TNFα, as well as inhibiting the synthesis of IL-2, IL-3, GM-CSF, TNFα and IFN-γ (D'Andrea *et al.*, 1993). IL-10 may contribute to disease severity through NS1-induced IL-10 production by monocytes, which in turn suppresses dengue-specific T cell responses (Adikari *et al.*, 2016; Malavige *et al.*, 2013b). Notably, data from animal studies suggest that NS1 activates immunological cascades in monocytes and macrophages leading to the pathologies observed in SD (Chen *et al.*, 2015; Modhiran *et al.*, 2015). In addition to its immunosuppressive roles, IL-10 has recently been reported as a protective mediator against plasma leakage and vascular dysfunction (Cheng & Sharma, 2015). IL-10 is secreted by a variety of cell types including $CD4^+$ and $CD8^+$ T cells, B cells, macrophages, monocytes, eosinophils and mast cells (O'Garra & Vieira, 2007). Measurements of circulating levels of IL-10 reflect the sum total of IL-10 produced from all cells in the body at any one point. Production of IL-10 may change according to the predominant cell type specific to the day of infection to regulate viral clearance immunopathology (reviewed by Tsai *et al.*, 2013b) as different immune cells are invoked at different stages of dengue infections (Boonnak *et al.*, 2008; Clyde *et al.*, 2006). The timing of IL-10 production is dynamic and varies throughout the disease course, exemplified by IL-10 levels peaking around early defervescence in DHF patients, but less so in DF patients (Adikari *et al.*, 2016; Butthep *et al.*, 2012; Kumar *et al.*, 2012; Libraty *et al.*, 2002a), suggesting that the biggest difference between DF and DHF patients may be observed around the day of early defervescence. Mechanistically, the intrinsic ADE of DENV infections may modulate the severity of dengue via increased IL-10 production and subsequent enhancement of the Suppressor of Cytokine Signalling (SOCS) system (Chareonsirisuthigul *et al.*, 2007; Suhrbier & La Linn, 2003; Ubol *et al.*, 2010). Furthermore, *in vitro* studies suggest that in ADE-DENV infections, the role of IL-10 changes in the early and later stages of infection from anti-viral to immunoregulation (Halstead *et al.*, 2010; Tsai *et al.*, 2013b). Two studies noted significant elevations in IL-10 and IFNγ in DHF and DSS patients compared to DF patients ($P<0.01$) (Butthep *et al.*, 2012; Nguyen *et al.*, 2004), whereas one study showed elevated IL-10 levels (DF 11.7±3.5 vs DHF 117.0±52.8 pg ml^{-1}; $P=0.03$) but decreased IFNγ in DHF compared to DF (DF 130.2±16.9 vs DHF 84.2±12.6 pg ml^{-1}; $P=0.01$) (Chen *et al.*, 2005). By contrast, three other studies reported increased IL-10 levels in DHF patients, but no difference in IFNγ between DF and DHF patients (Brasier *et al.*, 2012; Green *et al.*, 1999a; Houghton-Triviño *et al.*, 2010). Note that these studies had different sampling timings. In addition, we found no consistent trend of any potential interactions between IL-10 with the following cytokines: IL-2, IL-6, IL-12 and TNFα. This is congruent with other studies as cytokine changes are often mixed in dengue infections in different populations (Chaturvedi *et al.*, 2000), with the exception of IL-10 as it is shown in this systematic review. Collectively, the exact sampling timing of IL-10 may be a critical aspect in determining its maximal potential as a marker for SD prognosis.

The discrepancy of IFNγ in the five studies could be due to the rapid kinetics in its circulating levels, and depending on which infection day blood was sampled and analysed the IFNγ levels could be different (Green *et al.*, 1999a; Libraty *et al.*, 2002b; Nguyen *et al.*, 2004). Reports from humans and from animal models suggest that IFNγ controls infections through viral clearance and limiting virus replication (Costa *et al.*, 2012; Horras *et al.*, 2011; Pal *et al.*, 2014; Shresta *et al.*, 2004). Notably, dengue viral titres correlate with disease severity (Endy *et al.*, 2004; Vaughn *et al.*, 2000), but are plausibly influenced by DENV type and infection status (Duyen *et al.*, 2011). Levels of IFNγ can be attenuated by IL-10 through SOCS-3 blockage of STAT1-IFNγ receptor interaction in intrinsic ADE-DENV infection (Chareonsirisuthigul *et al.*, 2007; Ubol *et al.*, 2010). Indeed, the absence of IFNγ in a mouse model of dengue led to primary dengue-infection-induced lethality (Costa *et al.*, 2012). Thus, depending on the infection status and DENV type, in combination with timing of IL-10 production, IFNγ levels may be affected, leading to altered viral clearance, prolonged infection and consequently determining disease severity. IFNγ levels peak before defervescence in DHF patients and peak after defervescence in DF patients, because of this the maximum difference in the febrile phase of dengue suggests an advantage of using IFNγ as an early marker of SD. However, the implication and utility of IFNγ needs further study with properly defined sampling windows and DENV types taken into consideration.

To our knowledge, this is the first systematic review of immuno-modulating proteins as prognostic markers of SD sequelae. Two earlier published reviews did not apply and justify quality assessments (Chaturvedi *et al.*, 2000; Yacoub & Wills, 2014). This is worth highlighting as the restriction of studies according to a set of inclusion/exclusion criteria, and the introduction of quality assessments, retained studies with better-defined methodologies. Longitudinal studies, where daily measurements of studied immuno-modulating proteins are taken, may best capture the dynamic kinetics of these proteins in dengue. Although a few immuno-modulating proteins are potentially promising as prognostic markers, prospective observation cohort trials, such as the one by International Research Consortium on Dengue Risk Assessment, Management, and Surveillance (IDAMS; https://ClinicalTrials.gov; identifier: NCT01550016), may aid further endorsement of the utility of an acute phase prognostic marker for SD. In addition, immuno-modulating proteins, in reflecting the cardinal symptoms of SD, plasma leakage and thrombocytopenia, should be given research priority (Yacoub & Wills, 2014; Zapata *et al.*, 2014). One promising example is TNFα, which was reported to induce endothelial cell apoptosis (Chen *et al.*, 2007) and was also noted in this review as an elevated

Table 2. Quality assessment criteria matrix

Criterion	Score		
	0	1	2
Study design (control, DF, DHF)	Only control vs DF	Control vs DHF, Control vs DF or DF vs DHF	
Timing of sampling (days)	No or ambiguous description	Full description	Within acute phase of infection ($\leq$5 days)
Age	No or ambiguous description	Full description	
Data collection	No or ambiguous description	Case–control or retrospective cohort	Prospective cohort
Diagnostic criteria [WHO (1997) or WHO, (2009)]	No or ambiguous description	Full description	
Inclusion/exclusion criteria	No or ambiguous description	Full description	
Infection status	No or ambiguous description	Full description	
DENV type	No or ambiguous description	Full description	
Statistical analysis	No or ambiguous description	Full description	
Data statistics (*P* value provision)	No or ambiguous description	Full description	

cytokine in DHF. Other potential circulating markers of dengue severity, such as proteases (Koraka *et al.*, 2010; St John *et al.*, 2013; van de Weg *et al.*, 2014), soluble adhesion molecules (Cardier *et al.*, 2006) or metabolites (Cui *et al.*, 2013, 2016), may show potential as alternative soluble prognostic markers of SD, but face similar challenges in the extensive demands of large study cohorts. Capturing soluble factors that correlate and potentially foretell pathognomonic symptoms may not only increase the specificity and sensitivity of prognosing SD, but also avoid the non-specific 'cytokine storms' observed in other acute infectious diseases, such as influenza and malaria (Clark, 2007).

There were several limitations in our review process. First, there were inadequate reports on the details of patient demographics, DENV type and patients' infection status (primary, secondary or tertiary infection) in the studies. Inclusion of such information may be necessary to understand why results may be heterogeneous. Critically, sampling windows (days of illness from onset of fever) were at times not documented, or varied widely, sometimes even within the same study. Second, endpoint measurements of these studies varied substantially, differing in the types of immuno-modulating proteins under study. Not all studies investigated the same type of immuno-modulating proteins in relation to dengue severity. Furthermore, data presented in tables or figures in the published manuscripts rarely report concentration means and/or the range of the significantly different immuno-modulating proteins. This severely limited any statistical estimation of odds ratios, sensitivity and specificity of the prognosis marker candidates and prevents meta-analysis. Such wide variability in studies has been reported elsewhere (Potts & Rothman, 2008). Third, after strictly adhering to internal assessments of the quality of the primary studies, further moderation led to six studies being downgraded and one study being upgraded (Table S1). A study may have scored as high quality but may have been weighed down due to an unlisted criterion, for example, large variation in the studied cytokines (Chen *et al.*, 2006). Although this could potentially introduce bias, we included this empirical exercise to ensure retention or exclusion of studies that were under- or over-ranked but still added value to the review.

Conclusions

This systematic review provides the basis for future high-quality studies urgently required to identify key prognostic immuno-modulating protein markers of SD. They should be performed according to REMARK criteria (McShane *et al.*, 2005) to avoid the methodological deficiencies discovered in this systematic review, mostly with substantial heterogeneity in the study populations and prognostic endpoints. Studies in larger population sizes should reflect in detail the study population's age, infection status and DENV types, with standardization on first sample collection timing (<96 h from fever onset), detailed patient infection information and prognostic endpoint measurements.

The use of soluble immuno-modulating proteins as prognostic markers of SD remains under investigation and yet a consensus marker or marker signature for prognosing SD is desirable. Is this achievable? Current evidence suggests that different patient subpopulations – age groups (adults vs children vs geriatric), DENV types 1 to 4, and infection status – respond differently to dengue infections (Hammond *et al.*, 2005; OhAinle *et al.*, 2011; Tricou *et al.*, 2011). Accordingly, different markers may be needed to optimally prognose SD in different patient subpopulations (Lee & Ooi, 2013), and potentially aid the design of dengue vaccines.

Methods

Search strategy and data extraction

The primary literature search was conducted according to PRISMA (Preferred Reporting Items for Systematic Reviews

Table 3. Studies with significantly different IL-10 levels in dengue patients

Publication	Sample size		Age (years)		Infection status			Information on DENV type available?	First sampling time	DHF IL-10 change relative to DF
	DF	DHF	DF	DHF	DF/ DHF	Primary (%)	Secondary (%)			
Braiser *et al.* (2012)	38	13	15.76±7.8	19±13.4	DF	Not stated	Not stated	Yes, DF predominant DENV-1, DHF predominant DENV-2	Day 1 upon fever onset	Increase
					DHF	Not stated	Not stated			
Butthep *et al.* (2012)	51	98	Not stated	Not stated	DF	Not stated	Not stated	No	−3 days from defervescence	Increase
					DHF	Not stated	Not stated			
Chen *et al.* (2005)	66	33	46.8 (20–81)	57.8 (30–76)	DF	75	25	Yes, DENV-2	1–7 days post-symptom onset	Increase
					DHF	43	57			
Green *et al.* (1999b)	22	20	8.1 (3.2)*	8.1 (2.6)*	DF	41	10	Yes, DF predominant DENV-1, DHF predominant DENV-2	<72 h post-fever onset	Increase
					DHF	59	90			
Houghton-Triviño *et al.* (2010)	21	17	26 (3–55)	4 (0.3–12)	DF	23.8	76.2	Yes, DF and DHF predominant DENV-1	2–7 days of the disease	Increase
					DHF	11.8	88.2			
Malavige *et al.* (2013a)†	Non-SD: 219	SD: 40	Non-SD: 27.4 (11.4)	SD: 23.3 (8.8)	Non-SD	Not differentiated between non-SD and SD		No	4 and 5 days of fever	Increase
					SD					
Nguyen *et al.* (2004)‡	85§	22‖	6.4 (1–11)	8.2 (4–11)	DHF I/II	75.7	3.7	Partial	3–7 days post-fever onset	Increase
					DHF III/IV	19.6	0.9			
Pérez *et al.* (2004)	28	6	16–59 (not differentiated between DF and DHF)		DF	39	61	No	1–3 days post-fever onset, up to day 5	Increase
					DHF	0	100			

*The information provided here is a subset of patients described in Kalayanarooj *et al.* (1997).
†Comparison made between non-SD and SD, following the WHO (2009) case definition.
‡Comparison was DHF III/IV relative to DHF I/II.
§DHF I/II.
‖DHF III/IV (DSS).

Table 4. Studies with significantly different IFNγ levels in dengue patients

Publication	Sample size		Age (years)		Infection status			Information on DENV type available?	First sampling time	DHF IFNγ change relative to DF
	DF	DHF	DF	DHF	DF/DHF	Primary (%)	Secondary (%)			
Bozza *et al.* (2008)	20	39	28–48	23–53	DF DHF	74 58	26 42	Incomplete, mix of DENV1 and DENV-3	3–10 days after disease onset	Increase
Butthep *et al.* (2012)	51	98	Not stated	Not stated	DF DHF	Not stated Not stated	Not stated Not stated	No	−3 days from defervescence	Increase
Chen *et al.* (2005)	66	33	46.8 (20–81)	57.8 (30–76)	DF DHF	75 43	25 57	Yes, DENV-2	1–7 days post-symptom onset	Decrease
Nguyen *et al.* (2004)*	85†	22‡	6.4 (1–11)	8.2 (4–11)	DHF I/II DHF III/IV	75.7 19.6	3.7 0.9	Partial	3–7 days post-fever onset	Increased in DHF I–IV compared to healthy controls
Kumar *et al.* (2012)	44	18	39 (13.02)	40 (14.08)	DF DHF	56.8 61.1	43.2 38.9	Yes, DF and DHF predominant DENV-1	<72 h post-fever onset	Decrease
Soundravally *et al.* (2014)	27	30	22 (16–67)	35 (20–59)	DF DHF	Not stated Not stated	Not stated Not stated	Yes, DENV-3	4 or 5 days post-fever onset	Decrease

*Comparison was between healthy controls, DHF I/II and DHF III/IV.
†DHF I/II.
‡DHF III/IV (DSS).

Table 5. Electronic search strategy design

Databases and years searched	Date searched and search files	Number retrieved
PubMed (1965–current)	1 Dengue [all fields] (13517) 2 Dengue [MESH terms] (7753) 3 1 OR 2 (13517) 4 Cytokine OR Chemokine [Text Word] (190750) 5 Cytokine [MESH terms] (545522) 6 Cytokine OR Chemokine [Title/Abstract] (173026) 7 4 OR 5 OR 6 (595542) 8 Biological markers [MESH terms] (642853) 9 Biomarker* OR bio-marker* OR marker* [Text Word] (754080) 10 Biomarker* OR bio-marker* OR marker* [Title/Abstract] (619463) 11 8 OR 9 OR 10 (1060932) 12 7 OR 11 (1515594) 13 predict* OR prognos* OR correlate* OR associat* OR indicat* [Text Word] (6348997) 14 predict* OR prognos* OR correlate* OR associat* OR indicat* [Title/Abstract] (6401251) 15 12 OR 13 (6674213) 16 3 AND 12 AND 15 (596) 17 Filter: English AND Human (451)	451
Embase (1974–current)	1 Exp dengue (12608) 2 Cytokine or Chemokine or Biological Marker or (Marker* or biomarker* or bio-marker*).tw. (1014955) 3 predict* or prognos* or indicat* or associat* or correlat*.tw. (7851000) 4 1 AND 2 AND 3 (401) 5 Limit 5 to (human AND English language) (343)	343
	TOTAL (include duplicates)	**794**

and Meta-Analyses) guidelines (Moher *et al.*, 2009), via PubMed and Embase databases, for original research articles with restriction to human studies and English language. The search terms used for both databases were as followed: 'dengue AND (Cytokine OR Chemokine) AND (predict* OR prognos* OR correlat* OR associat* OR indicat*)'. We designed search strategies as shown in Table 5.

Two independent reviewers (W. Y. L. and Y. H. L.) screened the results to identify relevant literature based on titles and abstracts (when available), followed by another evaluation according to the inclusion and exclusion criteria. Articles with reported epidemiology, clinical signs, laboratory parameters and prognosis markers of SD outcome were included.

Our exclusion criteria included animal models, cell lines (*in vitro* studies), *ex vivo* cell studies, vaccine or anti-viral trials, genetic markers studies, studies without controls or inappropriate controls and also review articles. Of note, this review is devoted to the utility of human circulating cytokines and chemokines as prognostication markers; hence, other proteins such as immunoglobulins, acute phase proteins and proteases were excluded. When both reviewers (W. Y. L. and Y. H. L.) agreed on the final selected title and abstracts, the full texts of the articles were obtained and independently reviewed for eligibility.

Data extraction was performed by a single reviewer (W. Y. L.) using a data extraction sheet and was checked by another reviewer (Y. H. L.). The data extracted included the name of first author, year of publication, age, gender, disease outcome – DF/DHF/DSS or DwoWS, DwWS and SD – sample size, study design, location of study, specific markers investigated and results of the analysis.

Risk of bias and quality assessment

Two reviewers (W. Y. L. and Y. H. L.) independently evaluated the risk of bias in selected studies by assessing studies according to a checklist developed by the Cochrane NRS Methods group (Cochrane Group, 2012) as shown in Table S3. Assessment of outcome was not masked in all studies due to the need to diagnose dengue severity.

Subsequently, the quality of selected studies was assessed across a matrix of ten metrics, namely (i) study design, (ii) time of sampling, (iii) age, (iv) data collection, (v) diagnostic criteria, (vi) inclusion/exclusion criteria, (vii) infection status, (viii) DENV type, (ix) statistical analysis, (x) data statistics. The quality assessment score system was based on a modified version of the Newcastle–Ottawa scale (NOS) (Wells *et al.*, 2013) and is described in Table 2. The maximum score is 12, and a score of 9–12 is considered as high quality, 5–8 as moderate quality and 1–4 as low quality. The measure of agreement between the two reviewers (W. Y. L. and Y. H. L.) was assessed using the Kappa index (MedCalc version 12.5). None of the studies were randomized clinical trials and

because the studies were observational studies (case–control, cohort), we assessed the quality of evidence using a list of ten criteria modified from the NOS (Table 2), rather than Grading of Recommendations, Assessment, Development and Evaluations (GRADE) (Guyatt *et al.*, 2008).

Ascertainment of outcomes relied on WHO (1986), WHO (1997) or WHO (2009) dengue case definition guidelines. The WHO (1986) and WHO (1997) case definition guidelines were grouped together during analysis, but were analysed separately from the WHO (2009) case definition guidelines due to reported differences (Leo *et al.*, 2013; Narvaez *et al.*, 2011; Tsai *et al.*, 2013a). There was the possibility of bias or discrepancy in the classification of dengue severity; therefore, two reviewers (W. Y. L. and Y. H. L.) assessed the studies either for explicit description of the WHO guidelines used or for labelling of the groups' categorization (DF, DHF and DSS correspond to the WHO (1986) and WHO (1997) case definitions, whereas non-SD, SD, DwoWS and DwWS correspond to the WHO (2009) case definition).

Odds ratios or statistical mean/median values could not be identified in most studies. No pooling was done given the extra sources of methodological diversity (types of prognostic factors being measured) and bias. Hence, NRS are expected to be more heterogeneous than randomized trials.

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