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# Efficacy and safety of rituximab in autoimmune and microangiopathic hemolytic anemia: a systematic review and meta-analysis

Shih-Hsuan Chao<sup>1</sup>, Yuh-Lih Chang<sup>2</sup>, Jiin-Cherng Yen<sup>1</sup>, Hsien-Tzung Liao<sup>3</sup>, Tsai-Hung Wu<sup>4</sup>, Chia-Li Yu<sup>5</sup>, Chang-Youh Tsai<sup>3\*</sup>  and Yueh-Ching Chou<sup>2</sup>

## Abstract

**Background:** The efficacy and safety of rituximab (RTX) on hemolytic anemia (HA) is unknown. Therefore we retrospectively analyze the efficacy and safety of RTX in autoimmune hemolytic anemia (AIHA) and microangiopathic hemolytic anemia (MAHA) from the previous literature.

**Methods:** Data in clinical trials and observational studies were collected from PubMed, Cochrane, Embase, and Google Scholar until Oct 15, 2018. The efficacy and safety of RTX in patients with AIHA or MAHA were assessed and overall response rates (ORRs), complete response rates (CRRs), adverse events (AEs) and relapse rates (RRs) were extracted if available. A meta-analysis was performed with a random-effects model, estimating mean proportions in all studies, and relative rates in comparative studies.

**Results:** After quality assessment, a total of 37 investigations encompassing 1057 patients eligible for meta-analysis were included. Pooled mean proportion of ORR was 0.84 (95% confidence interval [CI] 0.80–0.88), and that of CRR was 0.61 (95% CI 0.49–0.73). Mean AE rate was 0.14 (95% CI 0.10–0.17), and mean RR was 0.21 (95% CI 0.15–0.26). Relative ORR was 1.18 (95% CI 1.02–1.36), and relative CRR was 1.17 (95% CI 0.98–1.39) fold more than the respective non-RTX counter parts. Relative AE rate was 0.77 (95% CI 0.36–1.63), and relative RR was 0.93 (95% CI 0.56–1.55) fold less than the respective non-RTX counter parts.

**Conclusion:** RTX is more effective than the treatments without RTX for AIHA and MAHA and is well-tolerated.

## Introduction

Hemolytic anemia (HA) is an anemia due to premature destruction of erythrocytes (or red blood cells, RBCs) in the circulation before their normal demise [1]. Its diagnosis is based on decreased hemoglobin and/or haptoglobin, increased reticulocytes, indirect bilirubin, and lactate dehydrogenase, as well as typical findings

demonstrated in peripheral blood smear [1, 2]. There are several causes of HA, which may result in erythrocyte destruction occurring at different locations, such as in larger vessels in the case of autoimmune hemolytic anemia (AIHA) or in smaller vessels in the case of microangiopathic hemolytic anemia (MAHA) [2].

AIHA is caused by autoantibodies against self-antigens in erythrocytes, leading to a premature RBC destruction, which could be diagnosed by a positive direct antiglobulin test (DAT) [3]. AIHA can be subdivided into warm, mixed, or cold-reactive subtypes, according to the optimal autoantibody-RBC reactivity

\*Correspondence: cytsai@vghtpe.gov.tw

<sup>3</sup> Division of Allergy Immunology & Rheumatology, Taipei Veterans General Hospital, 201 Shih-Pai Rd Sec 2, Taipei 112, Taiwan  
Full list of author information is available at the end of the article



temperatures [4–6]. Treatment options depend on the types of AIHA, with corticosteroids or supportive care as the mainstay of the treatment [7].

MAHA is a descriptive term for non-immune hemolysis resulting from intravascular RBC fragmentation [8]. Its main difference from AIHA is that patients with MAHA get negative in DAT [8]. The prominent causes of MAHA are thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) [8]. The disease can be hereditary or acquired by inhibiting autoantibodies [9]. TTP is mainly caused by a severely reduced activity of the von Willebrand factor-cleaving protease, ADAMTS13. Its standard treatment is plasma exchange (PEX) in conjunction with corticosteroids [10]. Typical infection-associated HUS is triggered by Shiga toxin producing *Escherichia coli*, and primary HUS is caused by complement dysregulation. The standard treatment is eculizumab for HUS [11].

Rituximab (RTX) is a B cell depleting monoclonal antibody which binds to cluster of differentiation (CD) 20 expressed on the surface of B cell that produces anti-RBC antibodies. It has been used as a therapeutic biologic agent in B cell lymphoma and leukemia [12]. A systematic review has discussed about safety and efficacy of RTX in immune-mediated disorders [13]. A meta-analysis has shown high short-term benefit/risk ratio of RTX in AIHA [14]. A randomized controlled trial (RCT) has revealed that RTX combined with prednisone may confer a better benefit/risk ratio than prednisone alone for treating adults with newly-diagnosed warm-type AIHA (wAIHA) [15]. A phase 2 study showed a high remission and survival rate for TTP patients treated with PEX and RTX [16]. Overall, RTX may be effective in AIHA or, presumably in MAHA. However, the evidence of favorability for this treatment modality is not yet clear. To further investigate the efficacy and safety of RTX in AIHA and MAHA, we conducted this systematic review and meta-analysis.

## Methods

### Data sources and search strategy

All stages of the present investigation followed the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) and Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines [17, 18]. An exhaustive literature search was carried out on PubMed, Cochrane Library, Embase, and Google Scholar from the inception to Oct 15, 2018. The search criteria included AIHA, MAHA, thrombotic microangiopathy (TMA), TTP or HUS and RTX in Medical Subject Heading (MeSH), limiting to human studies.

### Study selection

We included clinical trials or observational studies published in English, which assessed the efficacy and safety of RTX in AIHA or MAHA (TTP/HUS). Duplicated investigations, studies in other research scopes or without desired outcomes, enrollment with insufficient sample size of less than 10 patients, and those without full article publication were excluded.

### Data extraction

The extracted data included sample size, population, age, gender, dosage and framework of RTX treatment, the first author's name, year of publication, study design, time of first evaluation, mean follow-up time and definition of treatment efficacy. The number of patients with overall response (OR) and/or complete response (CR) was extracted from each study. When available, the eventual relapse, side effects or toxicities were also collected.

### Quality assessment

Quality was assessed using the revised and validated version of Methodological Index for Non-Randomized Studies (MINORS) [19]. Non-comparative studies were scored on clearly stated aim, inclusion of consecutive patients, prospective collection of data, appropriate endpoints, unbiased assessment of the study endpoint, appropriate follow-up time period, loss to follow-up of less than 5%, and prospective calculation of the study size. Additional criteria for comparative studies were an adequate control group, contemporary control groups, baseline equivalence of groups, and adequate statistical analyses. Studies received 0 to 2 points for each of these components. The total score ranged from 0 to 24 points for comparative studies and 0 to 16 points for non-comparative studies. Scores no less than 18 in comparative studies and no less than 12 in non-comparative studies were considered to be with high quality and were included in quantitative analysis.

### Data synthesis and analysis

Efficacy was assessed by overall response rate (ORR) and complete response rate (CRR). Weighted mean proportions of response rate were calculated over all studies that included in the quantitative analysis, and relative ORR and relative CRR were calculated over comparative studies. As for the safety, adverse events (AEs) were extracted from all included studies. The weighted mean relapse rate (RR) in all studies and relative RR in comparative studies were calculated. We used a random-effects model to control the between-study variance and to produce an overall

summary as described by Dersimonian and Laird [20]. R Software (package metafor, version 2.0-0) was used for all analyses.

We assessed statistical heterogeneity by using  $I^2$  statistic and Cochran's Q test. An  $I^2$  statistic with values greater than 50% or Q test with  $p$ -value less than 0.1 indicates substantial heterogeneity. To explain heterogeneity, the effects of covariates on response rate were investigated using mixed-effects meta-regression. The risk of publication bias was determined by the method of funnel plot and Egger's regression test, which plots the natural log of effect sizes versus their standard error, and linear regression of funnel plot test asymmetry.

## Results

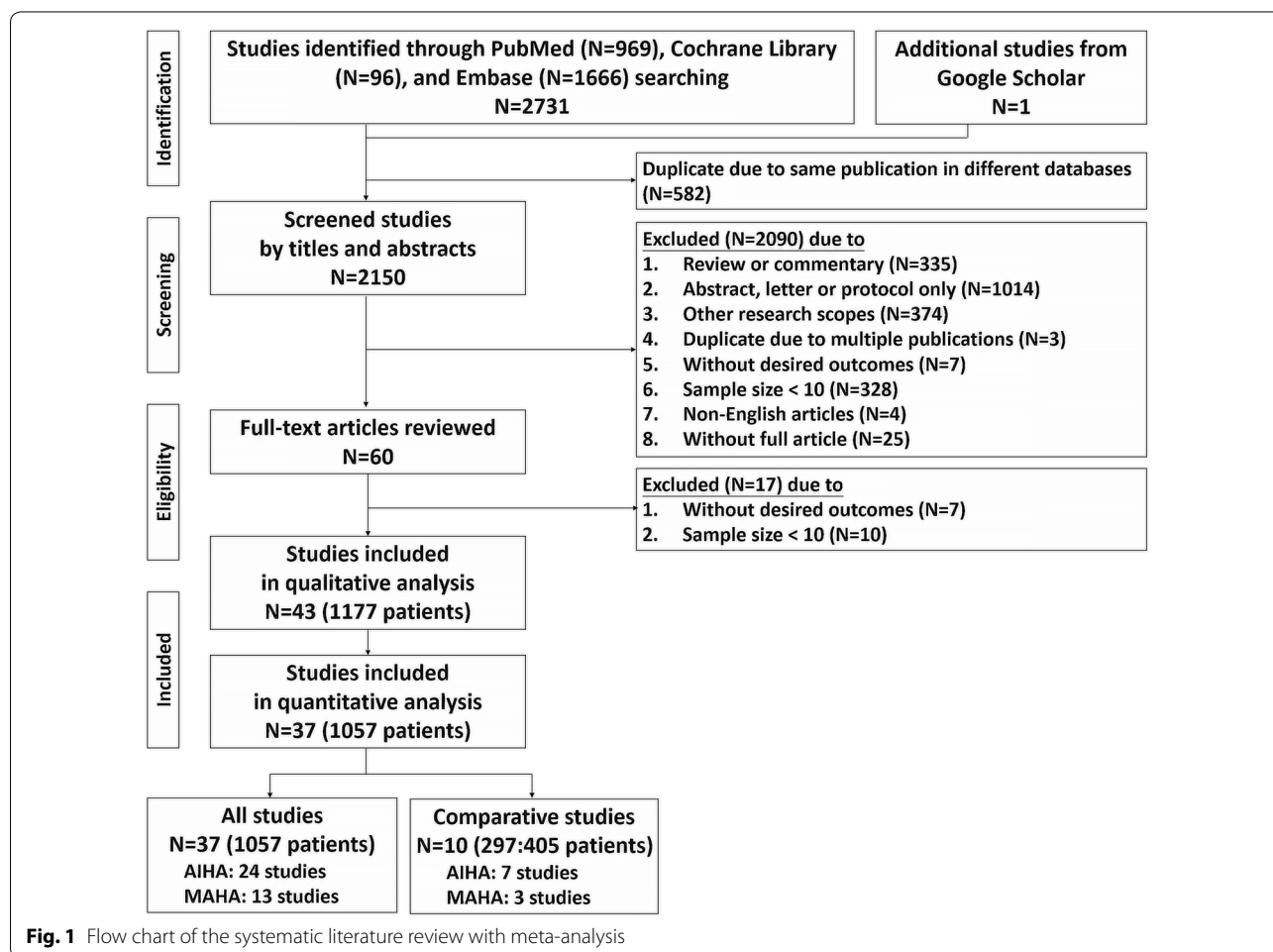
### Study selection

Figure 1 summarized the flow chart of the systematic literature review with meta-analysis. A total of 2732 articles were initially identified through electronic searching. After duplication of the same publications in different databases were removed, the titles and abstracts of the

remaining studies were reviewed, and 60 articles with potentially relevant studies were further identified in full-text. Finally, 43 published studies were determined to be eligible and were included in the qualitative analysis. Among 43 studies included in the qualitative analysis, 37 studies reached the cut point of high quality and were included in the quantitative analysis [15, 16, 21–55].

### Description of the studies

Characteristics of the included studies are shown in Table 1. Among 37 studies included in the quantitative analysis, 24 studies dealt with AIHA, while 13 included MAHA patients. Most of them were observational studies, and 10 of them were comparative studies, encompassing 7 AIHA studies and 3 MAHA studies. Sample size varied from 10 to 86 patients, the mean age was 50.6 years old, and the mean proportion of male was 41.0%. Patients mostly received four weekly infusions of RTX at 375 mg/m<sup>2</sup>. Time of the first evaluation was 0.2 month to 1 year, and the follow-up time ranged from 6 to 83 months.



**Fig. 1** Flow chart of the systematic literature review with meta-analysis

**Table 1 Characteristics of studies included in quantitative analysis**

Disease	AIHA	MAHA
All studies	24 studies	13 studies
37 studies	746 patients	311 patients
1057 patients		
	wAIHA	TTP
	8 studies	12 studies
	182 patients	297 patients
	CAD	HUS
	5 studies	1 study
	173 patients	14 patients
Comparative studies	7 studies	3 studies
10 studies	232:303 patients	65:102 patients
297:405 patients		

Publication year: 2004–2018; sample size: 10–86 (mean age: 50.6, mean male proportion: 41.0%); rituximab regimen: 28 with 375 mg/m<sup>2</sup> 4 weekly, 8 with other regimen (e.g. 100 mg 4 weekly); study design: 13 clinical trials, 24 observational studies; 1st evaluation time: 0.2 months–1 year; follow-up time: 6–83 months

### Efficacy in all studies

After pooling the effect of 37 studies, we got the weighted mean proportion of response rate. The ORR was 84% (95% CI 0.80–0.88) with high heterogeneity ( $I^2=75.76%$ ,  $p<0.01$ ), as shown in Fig. 2a. Funnel plot of ORR was asymmetrical as shown in Fig. 3a, which indicated possible publication bias (Egger's regression test,  $p<0.0001$ ). The CRR was 61% (95% CI 0.49–0.73) with significant heterogeneity ( $I^2=97.46%$ ,  $p<0.01$ ), as shown in Fig. 2b. Funnel plot of CRR was symmetrical, indicating no publication bias (Fig. 3b, Egger's regression test,  $p=0.631$ ).

Subgroup analyses were done because of the high heterogeneity, which were shown in Table 2 in detail. For AIHA, ORR was 79% (95% CI 0.73–0.84) and CRR was 44% (95% CI 0.33–0.55). Eight studies provided data for wAIHA with ORR of 79% (95% CI 0.69–0.90) and CRR was 49% (95% CI 0.22–0.77). For cold agglutinin disease (CAD), ORR was 66% (95% CI 0.57–0.76) and CRR was 14% (95% CI 0.03–0.25). As for MAHA, the response rate was 93% (95% CI 0.89–0.97). Further dividing MAHA into TTP and HUS, the response rate was 92% (95% CI 0.88–0.96) in TTP and 97% (95% CI 0.88–1.06) in HUS respectively. There was no significant difference wherever these data came from with regards to the study design or dosage regimen. All treatment modalities in MAHA studies were in combination with PEX or plasma infusion. Therefore, we couldn't analyze the impact of different treatment modalities in MAHA studies. But the response rate of RTX alone were similar to combination therapy in AIHA (RTX alone: 82% in OR, 44% in CR; treatments in combination: 74% in OR, 45% in CR).

We further carried out meta-regression to find out another factors that may influence the response rate

(Table 3). Among all the factors we tested, ORR and CRR were only significantly associated with age (ORR:  $r^2=23.29$ ,  $p=0.0103$ ; CRR:  $r^2=45.37$ ,  $p<0.0001$ ) as shown in Fig. 4a, b, respectively.

Among 872 patients included, there were 163 relapse events, with an overall RR of 21% (95% CI 0.15–0.26) (Fig. 2c). Because funnel plot for RR was asymmetrical (Fig. 3c), it is possible that there is a publication bias (Egger's regression test,  $p<0.0001$ ). In a further subgroup analysis (Table 2), RR was 28% (95% CI 0.20–0.36) in AIHA and 9% (95% CI 0.05–0.13) in MAHA respectively. However, meta-regression failed to demonstrate any other additional confounding factor (Table 3).

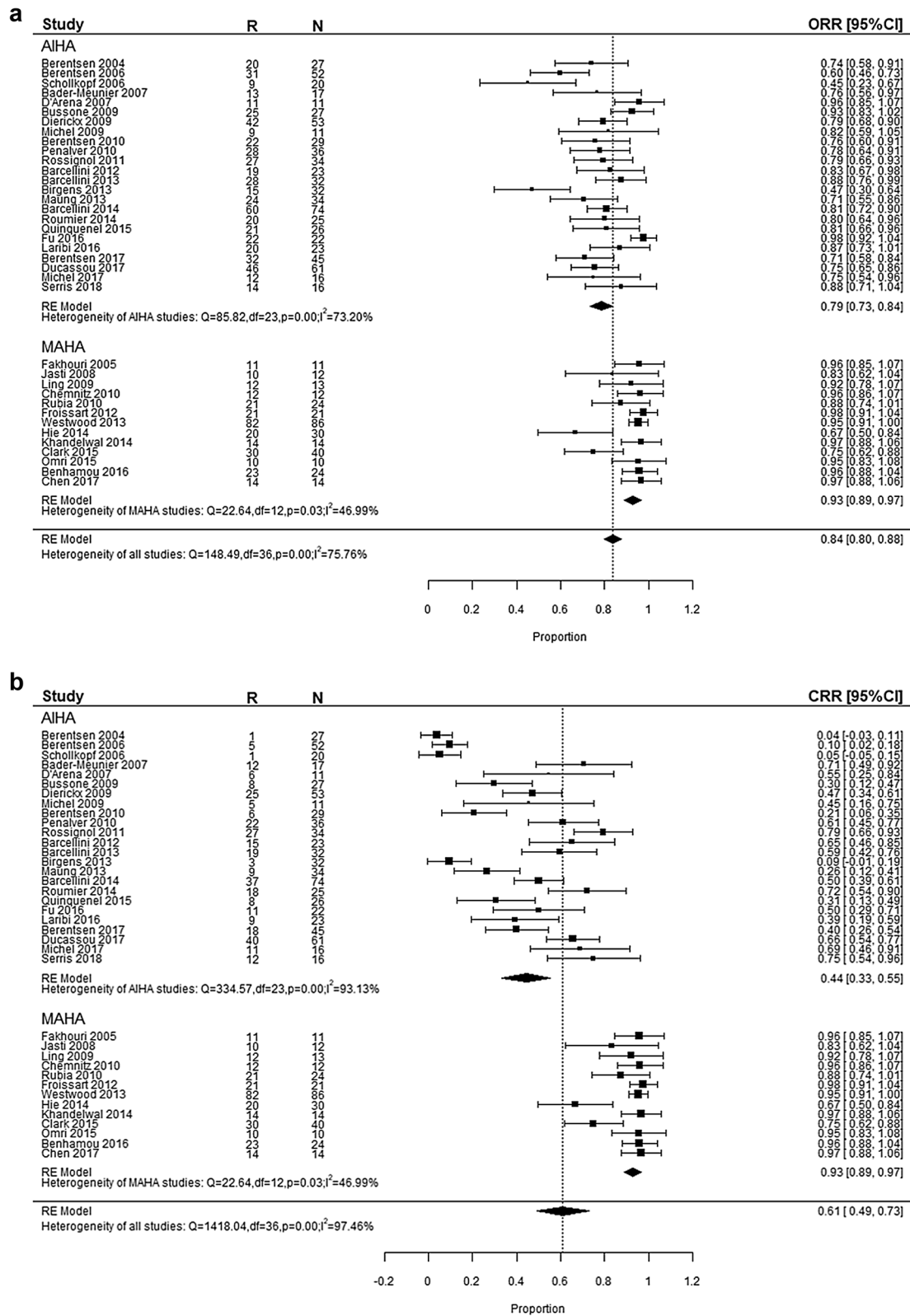
### Safety in all studies

Briefly, 32 studies reported 138 AEs in 851 patients. The overall AE rate was 13% (95% CI 0.10–0.17). Among them, 33 events (23.9%) were infusion-related reactions, such as fever, chills, skin rash, and allergic reactions, 34 events (24.6%) were infections, and 40 events (29.0%) were hematologic abnormalities, mostly neutropenia. Besides, there were 3 mortalities (0.3%), including one early death from uncontrolled hemolysis [38]. The 2nd fatal patient died because of delayed treatment [55]. The 3rd fatal patient died shortly after RTX therapy, which was also claimed to be partially contributing to the death [42]. Importantly, there is a critical issue about hepatitis B virus reactivation (HBVr) regarding AE of RTX. There was no records about it in the present meta-analysis. The previous epidemiological study provided prevalence estimates for a mean global HBsAg prevalence of 4.9% [56]. In Taiwan, the mean HBsAg prevalence goes to 13.7% [56]. So, rationale for the absence of HBVr as AE in the present investigation may be that the included studies were all from Western countries. It is conceivable that quite different conclusion might be reached if the studies are conducted using Taiwanese data, which will be interesting.

### Efficacy in comparative studies

After pooling the effect of 10 comparative studies, we got the relative response rate. As shown in Fig. 2d, relative OR was 1.18 (95% CI 1.02–1.36) with high heterogeneity ( $I^2=72.10%$ ,  $p<0.01$ ). Funnel plot for relative OR was asymmetrical as shown in Fig. 3d, indicating possible publication bias (Egger's regression test,  $p=0.0056$ ). On the other hand, relative CR was 1.17 (95% CI 0.98–1.39) with heterogeneity ( $I^2=51.98%$ ,  $p=0.03$ , Fig. 2e) and with a symmetrical funnel plot indicating the absence of publication bias (Egger's regression test,  $p=0.1736$ , Fig. 3e).

Sensitivity analysis was carried out via leave-one-out approach in both relative ORR and relative CRR. In the



**Fig. 2** Forest plot. **a** Overall response rate of rituximab in AIHA and MAHA. **b** Complete response rate of rituximab in AIHA and MAHA. **c** Relapse rate of rituximab in AIHA and MAHA. **d** Relative overall response rate of rituximab in AIHA and MAHA. **e** Relative complete response rate of rituximab in AIHA and MAHA. **f** Relative relapse rate of rituximab in AIHA and MAHA

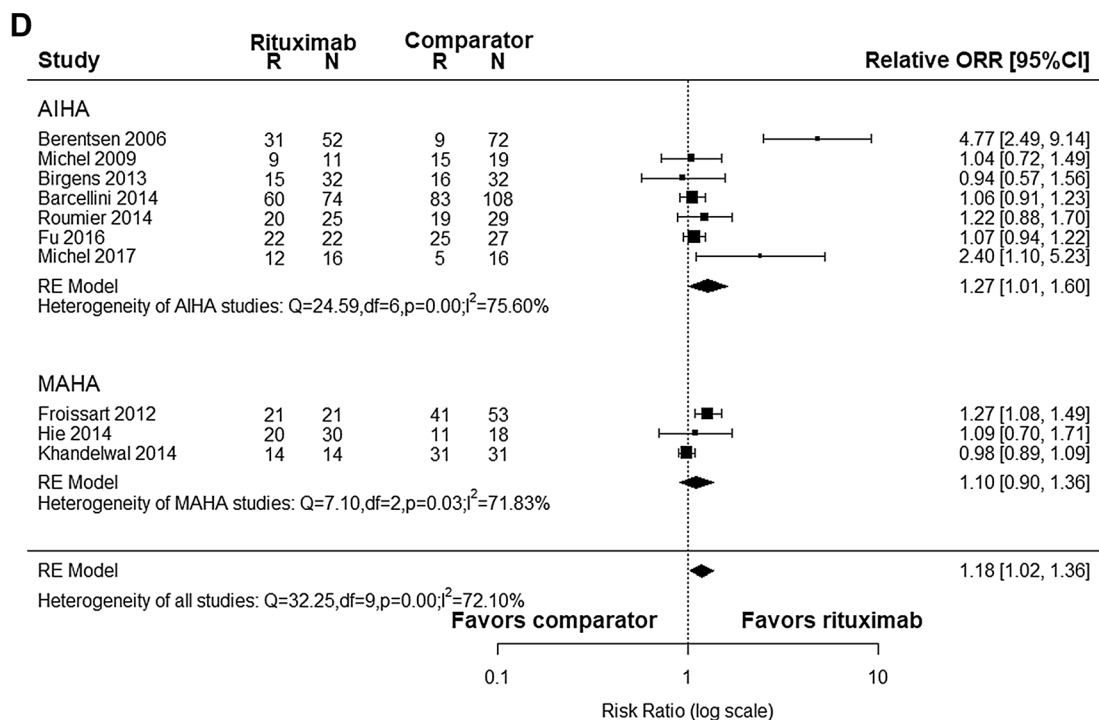
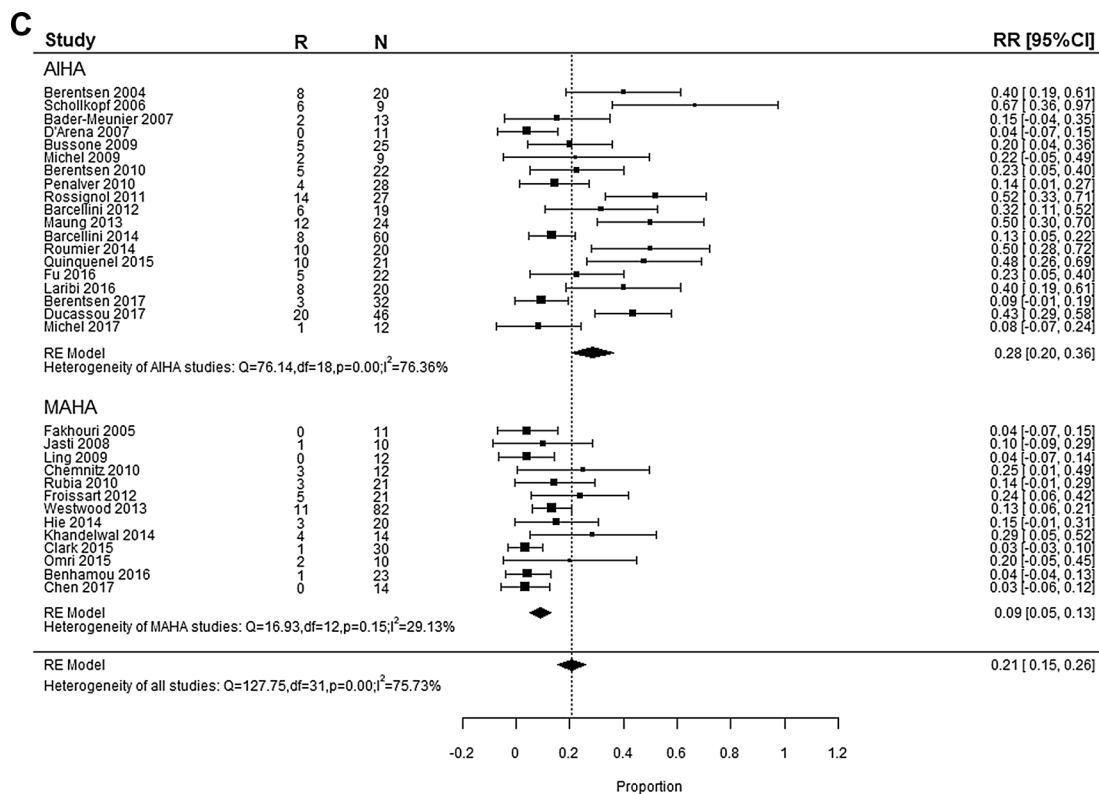


Fig. 2 continued

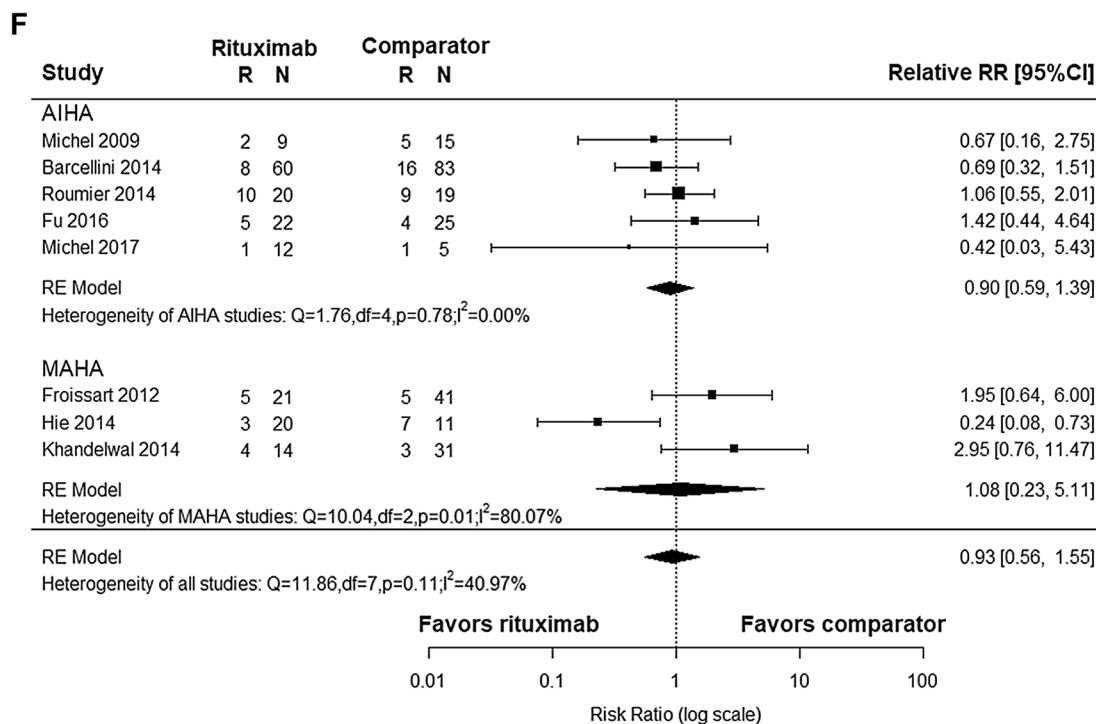
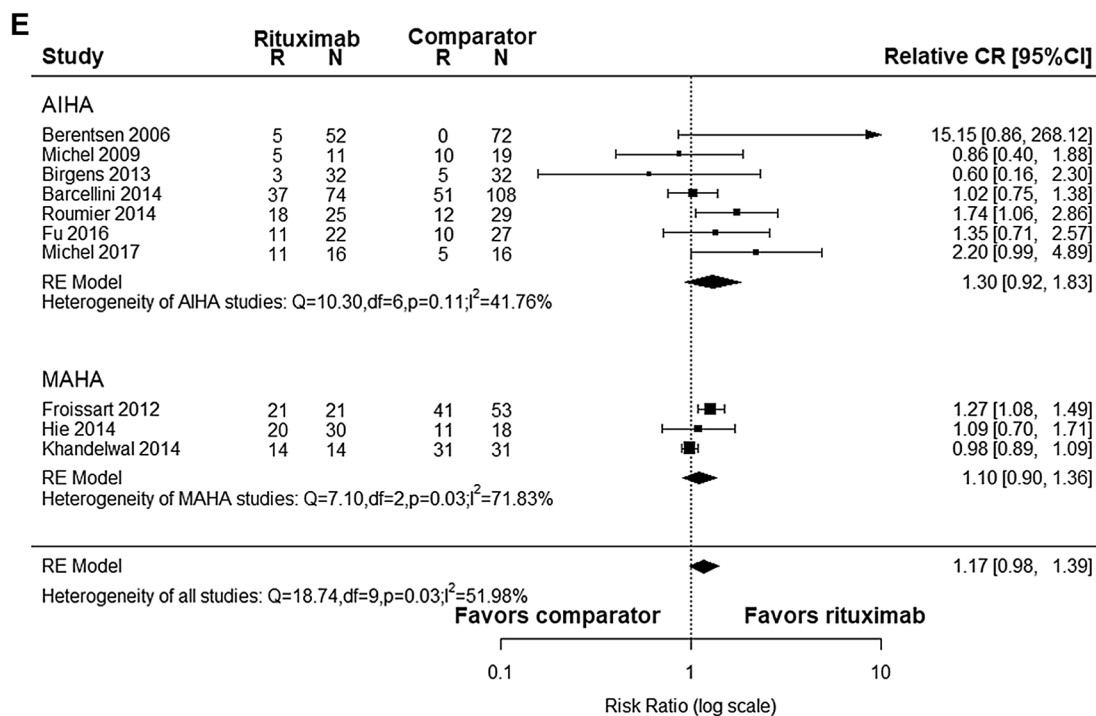
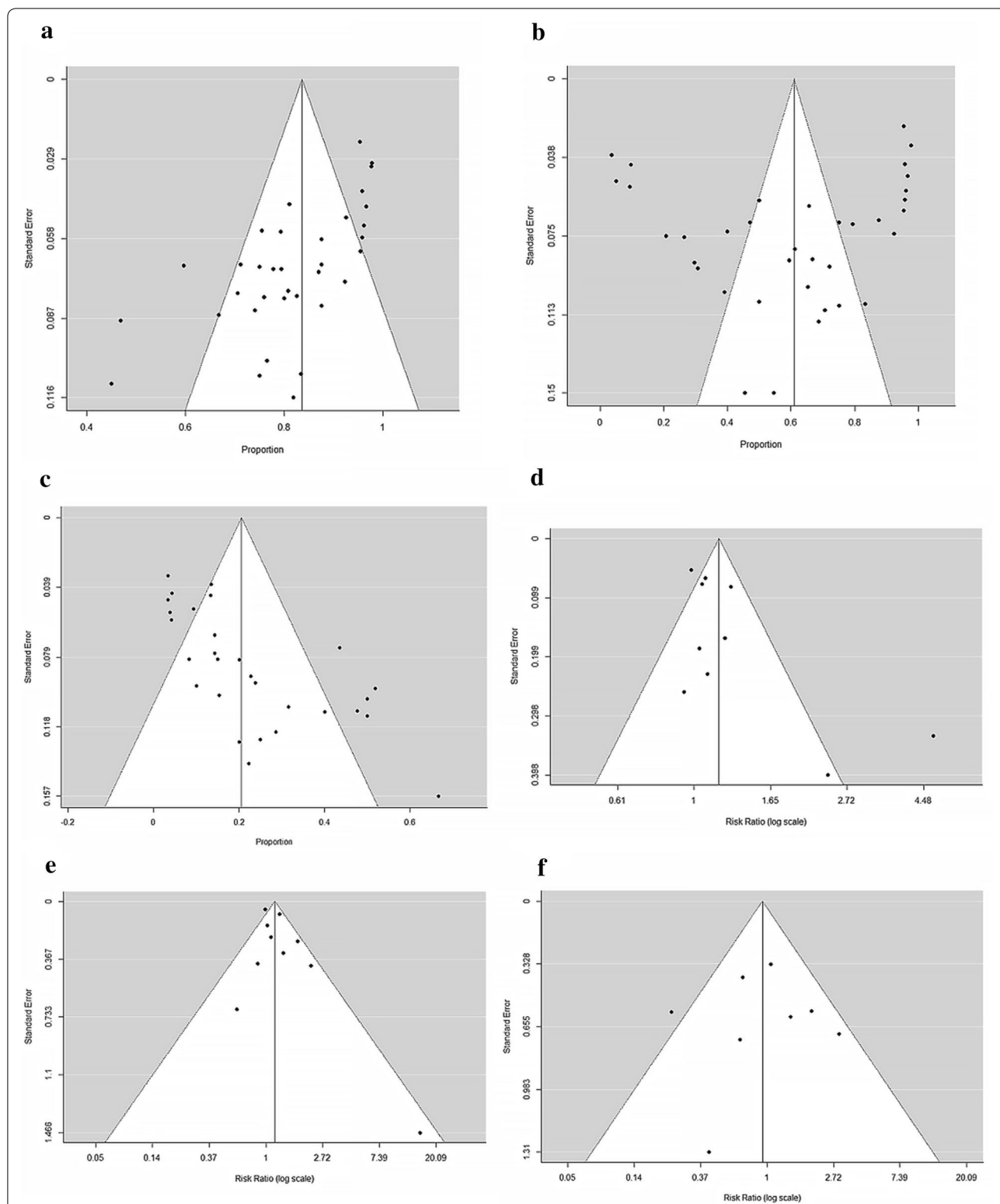


Fig. 2 continued



**Fig. 3** Funnel plot. **a** Publication bias of overall response rate of rituximab in AIHA and MAHA. **b** Publication bias of complete response rate of rituximab in AIHA and MAHA. **c** Relapse rate of rituximab in AIHA and MAHA. **d** Publication bias of relative overall response rate of rituximab in AIHA and MAHA. **e** Publication bias of relative complete response rate of rituximab in AIHA and MAHA. **f** Publication bias of relative relapse rate of rituximab in AIHA and MAHA



**Table 2 Subgroup analysis**

Subgroup category	OR rate			CR rate			RR		
	Study, n	ORR (95% CI)	I <sup>2</sup> (%)	Study, n	CRR (95% CI)	I <sup>2</sup> (%)	Study, n	RR (95% CI)	I <sup>2</sup> (%)
Overall	37	0.84 (0.80, 0.88)	75.76	37	0.61 (0.49, 0.73)	97.46	32	0.21 (0.15, 0.26)	75.73
Study design									
Clinical trial	13	0.80 (0.72, 0.89)	83.81	13	0.56 (0.32, 0.80)	98.54	11	0.15 (0.07, 0.22)	73.10
Observational study	24	0.85 (0.80, 0.89)	69.89	24	0.64 (0.51, 0.76)	95.79	21	0.23 (0.17, 0.30)	72.29
Diagnosis									
AIHA	24	0.79 (0.73, 0.84)	73.20	24	0.44 (0.33, 0.55)	93.13	19	0.28 (0.20, 0.36)	76.36
wAIHA	8	0.79 (0.69, 0.90)	75.89	8	0.49 (0.22, 0.77)	96.27	7	0.31 (0.14, 0.47)	82.22
CAD	5	0.66 (0.57, 0.76)	45.99	5	0.14 (0.03, 0.25)	82.57	4	0.31 (0.10, 0.53)	81.81
MAHA	13	0.93 (0.89, 0.97)	46.99	13	0.93 (0.89, 0.97)	46.99	13	0.09 (0.05, 0.13)	29.13
TTP	12	0.92 (0.88, 0.96)	50.74	12	0.92 (0.88, 0.96)	50.74	12	0.08 (0.04, 0.12)	21.38
HUS	1	0.97 (0.88, 1.06)		1	0.97 (0.88, 1.06)		1	0.29 (0.05, 0.52)	
Dosage regimen									
375 mg/m <sup>2</sup> 4 weekly	28	0.83 (0.78, 0.88)	78.64	28	0.62 (0.48, 0.77)	98.03	24	0.20 (0.14, 0.26)	77.50
Other regimen	8	0.84 (0.76, 0.91)	67.49	8	0.56 (0.45, 0.67)	70.80	7	0.24 (0.13, 0.32)	72.87
Treatment modalities of AIHA									
RTX alone	14	0.82 (0.76, 0.88)	72.74	14	0.44 (0.29, 0.59)	93.22	12	0.30 (0.20, 0.40)	78.27
In combination	10	0.74 (0.66, 0.81)	59.22	10	0.45 (0.27, 0.63)	93.70	7	0.26 (0.12, 0.39)	76.19

**Table 3 Meta-regression**

	OR rate			CR rate			RR		
	Study (n)	R <sup>2</sup> (%)	p-value	Study (n)	R <sup>2</sup> (%)	P-value	Study (n)	R <sup>2</sup> (%)	p-value
Sample size	37	5.11	0.1079	37	1.29	0.2262	32	0.00	0.7044
Year of publication	37	0.00	0.3522	37	5.83	0.0905	32	0.00	0.9247
Age	30	23.29	0.0103	30	45.37	<0.0001	27	0.00	0.3689
Proportion of male	30	0.00	0.6095	30	0.00	0.5294	27	0.00	0.4814
Evaluation time	29	3.70	0.2370	29	0.00	0.4967	27	0.00	0.6003
Follow-up time	37	8.59	0.1167	37	0.00	0.9396	32	0.00	0.6639
Quality score	37	0.00	0.1886	37	0.10	0.3369	32	0.42	0.3695

former, leaving “Berentsen et al. 2006” out resulted in the reduction in the overall heterogeneity significantly, coming to an ORR of 1.09 (95% CI 1.00–1.19). On the other hand, leaving “Khandelwal et al. 2014” out led to a reduction in overall CRR heterogeneity significantly, coming to 1.24 (95% CI 1.03–1.49).

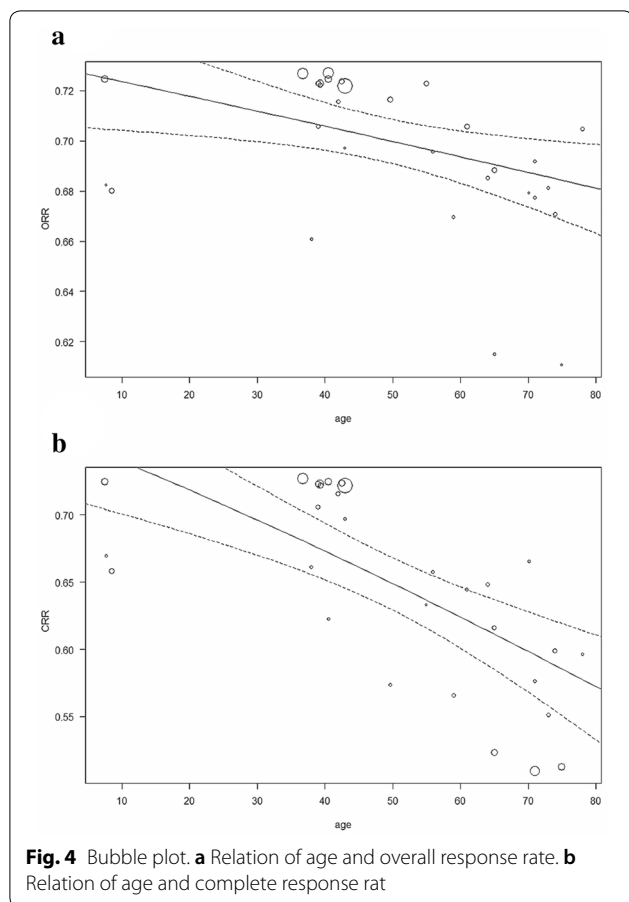
Among 10 comparative studies, 8 studies reported RR. The overall relative RR was 0.93 (95% CI 0.56–1.55, Fig. 2f) with a symmetrical funnel plot (Egger’s regression test,  $p=0.8543$ , Fig. 3f), indicating that there were no publication bias. Regarding the sensitivity analysis (Table 4), leaving “Hie et al. 2014” out resulted in reduction in the overall heterogeneity significantly, coming to 1.09 (95% CI 0.74, 1.60).

### Safety in comparative studies

Among 10 comparative studies, 6 studies documented AEs in both groups. In RTX group, there were 34 AEs in 130 patients, and there were 55 AEs in 188 patients in comparative group. The relative AE rate was 0.74 (95% CI 0.33–1.63) for RTX.

### Discussion

The results in the present “efficacy in AIHA study” were consistent with those reported previously [14]. However, our “efficacy in wAIHA” was higher than those reported in cold agglutinin disease (CAD). This implies that warm type AIHAs are more likely to be resulted from B cell hyperactivity. Moreover, the present study on “the efficacy



of RTX in MAHA” is the first one to our knowledge. We have found that the response rate of RTX was higher in MAHA. Furthermore, these effects were the same in patients either with TTP or HUS. The reason for this is unclear because apparently MAHA is not developing on the basis of deranged B cell regulation. However, HUS or TTP has been reported to be originated from autoantibodies such as ant-dsDNA, anticardiolipin antibodies, &/ or other autoantibodies against ADAMS13 [57, 58].

In subgroup analysis, regimens with different dosage of RTX showed a similar response rate. Most studies used the regimen of 375 mg/m<sup>2</sup> 4-weekly, which might contribute to the effects. These results are interesting because monoclonal antibody drugs are quite different from traditional small molecule drugs in that they are acting on the cells via the receptors or ligands. Since the cells usually have their own threshold or affinity limitation, the traditional dose–response relationship cannot be applied to this scenario. Moreover, the initial effect of the biologics on the cell can even be amplified or reduced via cell–cell contact or crosstalk. Further investigations may be necessary to determine the optimal dosage for RTX in AIHA and MAHA. When it comes

to different treatment modalities, response rate of RTX alone were similar to combination therapy in AIHA, i.e., RTX alone can be reasonably considered in patients with AIHA. Furthermore, regarding the effect of age on the response rate of RTX in HA patients, we found that the response rate was significantly reduced along with aging of the recipients. It may imply that senility is aligned with degeneration of the immunity such that the immune therapy on B cells is also turning to be ineffective. However, it is also possible that younger patients present with a more benign form of the disease. As for RR, our result in AIHA (28%) were consistent with those from previous meta-analysis (27%) [14]. Patients with MAHA exhibited lower RR (9%) in the present study. Although there were some patients with relapse in this group, most of them recovered after repeating with RTX.

RTX seems safe and well-tolerated in AIHA and MAHA. This was consistent with previous meta-analysis [14]. Few AEs have been reported (13%) in all of our included studies and even when they were present, the majority of them were otherwise mild. However, there are some common AEs in the in-label use of RTX, which were more than 25% in incidence, such as infusion reactions. Overall, RTX showed lower AE rate in AIHA and MAHA than in other diseases.

On the other hand, there is a critical issue about hepatitis B virus reactivation (HBVr) regarding AE of RTX. Indeed, there was no records for this in the present meta-analysis. In the epidemiological studies, the average global prevalence of HBV infection is 4.8% whereas the figure is as high as 23.7% in Taiwan, which is among the highest in the Asia–Pacific region [55]. The absence of HBV related AE in the present meta-analysis is conceivable because the included studies were all carried out in Western countries. If the studies done in Asia–Pacific region including Taiwan were included, the results are expected to be quite different, necessitating further relevant investigations.

The present investigation has provided overwhelming evidence for the first time that RTX is effective for AIHA and MAHA, compared to the conventional treatment modalities that did not include RTX. A previous meta-analysis only estimated the pooled mean response rate of RTX in AIHA [14]. In sensitivity analysis of ORR, getting rid of Berentsen’s data in 2006 could reduce the overall heterogeneity significantly [22]. This is because they used a quite different comparators to assess the effect of RTX. Similarly, in sensitivity analysis of CRR, getting rid of Khandelwal’s data in 2014 resulted in a significant reduction of the overall heterogeneity [52]. The cause of it may be that their data only included HUS. Nevertheless, the response rate still favored RTX in both MAHA and AIHA.

**Table 4 Sensitivity analysis**

No.	Study that leave	OR rate			CR rate			RR		
		Relative rate (95% CI)	I <sup>2</sup> (%)	p-value	Relative rate (95% CI)	I <sup>2</sup> (%)	p-value	Relative rate (95% CI)	I <sup>2</sup> (%)	p-value
A4	Berentsen (2006)	1.09 (1.00, 1.19)	34.44	0.14	1.15 (0.98, 1.35)	48.37	0.05	0.96 (0.55, 1.71)	48.42	0.07
A12	Michel (2009)	1.20 (1.02, 1.40)	75.15	0.00	1.19 (0.99, 1.43)	56.56	0.02			
A18	Birgens (2013)	1.20 (1.03, 1.39)	74.94	0.00	1.18 (0.99, 1.41)	55.56	0.02			
A20	Barcellini (2014)	1.22 (1.02, 1.45)	75.07	0.00	1.21 (0.98, 1.48)	56.95	0.02	0.99 (0.54, 1.83)	46.25	0.08
A21	Roumier (2014)	1.18 (1.01, 1.37)	74.80	0.00	1.12 (0.95, 1.33)	47.14	0.06	0.90 (0.47, 1.73)	48.42	0.07
A23	Fu (2016)	1.22 (1.02, 1.46)	75.17	0.00	1.16 (0.97, 1.40)	56.23	0.02	0.88 (0.49, 1.56)	46.94	0.08
A27	Michel (2017)	1.15 (1.00, 1.32)	71.68	0.00	1.13 (0.96, 1.34)	48.94	0.05	0.96 (0.56, 1.65)	47.72	0.07
M7	Froissart (2012)	1.16 (0.99, 1.37)	71.24	0.00	1.15 (0.93, 1.41)	40.60	0.10	0.83 (0.49, 1.56)	39.83	0.13
M9	Hie (2014)	1.19 (1.02, 1.38)	75.20	0.00	1.18 (0.98, 1.44)	57.31	0.02	1.09 (0.74, 1.60)	0.00	0.47
M10	Khandelwal (2014)	1.23 (1.04, 1.46)	70.04	0.00	1.24 (1.03, 1.49)	25.52	0.22	0.83 (0.50, 1.35)	32.18	0.18

As for the RR, there were no differences between treatments with and without RTX. In sensitivity analysis, getting rid of Hie's data in 2014 resulted in reduction in the overall heterogeneity significantly. Their investigation was the only study significantly favoring rituximab with low RR. The follow-up time of their data was the longest (5 years), which has indicated that the long-term effect of RTX is better than that of standard cares. However, the RRs were still not different between two groups after leaving "Hie et al. 2014" out.

In the analysis of comparative studies, the AE rates were not different between treatments with and without RTX. Taken together, the safety of RTX seemed similar to that of the conventional treatment modalities.

In spite of the significant findings demonstrated in the present meta-analysis, there were still several limitations in it. Firstly, although we have searched extensively in the 4 databases for all the relevant studies, we could only found 2 randomized controlled trials. Although this has been inevitable because AIHA and MAHA are rare diseases in real life, it may cause a lower level of evidence. Secondly, our overall results showed high heterogeneity. To figure out the reasons for this, we carried out meta-regression and found that age might have been the factor that influences the response rate. The various comparators in comparative studies may have also contributed. Lastly, although our analytic process followed the MOOSE and PRISMA guidelines, publication bias existed in the estimation of ORR and RR. There are several reasons to result in the publication bias. For example, inclusion criteria in a meta-analysis may be biased if the selection is restricted to published trials or to trials published in English language journals [59]. On the other hand, most research with published bias lack studies with poor results, since studies with poor results are most likely not to be published. It may cause overestimation of the pooled effects. However, the studies that are lacking in our research are studies with better results, i.e., the lack of those studies in our research may only cause further underestimation of the effect. Hence, our results should have been convincing.

## Conclusion

RTX shows a high overall effect (84%) in AIHA and MAHA, and an even higher effect (93%) in MAHA subgroup. It also shows a better effect in both AIHA and MAHA in comparison with other treatment modalities. Moreover, the RR of the diseases after RTX treatment might have been low. Finally, RTX is safe and well-tolerated for the treatment of HA. Few AEs have been reported with most of them being mild, and the AE rate was similar to those reported in other treatment modalities.

## Abbreviations

ADAMTS13: A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; AE: Adverse event; AIHA: Autoimmune hemolytic anemia; CAD: Cold agglutinin disease; CD: Cluster of differentiation; CRR: Complete response rate; DAT: Direct antiglobulin test; HA: Hemolytic anemia; HUS: Hemolytic-uremic syndrome; MAHA: Microangiopathic hemolytic anemia; MeSH: Medical Subject Headings; MINORS: Methodological Index for Non-Randomized Studies; MOOSE: Meta-analysis Of Observational Studies in Epidemiology; ORR: Overall response rate; PRISMA: Preferred Reporting Items for Systematic reviews and Meta-Analyses; RBC: Red blood cell; RR: Relapse rate; RTX: Rituximab; TMA: Thrombotic microangiopathy; TTP: Thrombotic thrombocytopenic purpura; vWF: von Willebrand factor; wAIHA: Warm-reactive autoimmune hemolytic anemia.

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## Disclaimer

The views expressed are those of the author(s) and not necessarily those of the MOST or Taipei VGH.

## Authors' contributions

SHC, YCC, and YLC performed the systematic literature review under the supervision of CYT. SHC and YCC carried out the study. All authors participated in the discussion for the interpretation of results. YLC organized and chaired the meetings, along with YCC and JCY. SHC wrote the draft manuscript. CYT and HTL gave critical reviews and revision of the manuscript. All contents of the article has been approved by all coauthors. All authors read and approved the final manuscript.

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## Availability of data and materials

All data generated or analyzed during this study are included in the manuscript.

## Ethics approval and consent to participate

This is not applicable for this review.

## Consent for publication

We declared that the manuscript is original, has not been published before, and is not currently being considered for publication elsewhere.

## Competing interests

We declared that we have no competing interests.

## Author details

<sup>1</sup> Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan.

<sup>2</sup> Department of Pharmacy, Taipei Veterans General Hospital, Taipei, Taiwan.

<sup>3</sup> Division of Allergy Immunology & Rheumatology, Taipei Veterans General Hospital, 201 Shih-Pai Rd Sec 2, Taipei 112, Taiwan. <sup>4</sup> Division of Nephrology, Taipei Veterans General Hospital, Taipei, Taiwan. <sup>5</sup> Division of Rheumatology Immunology & Allergy, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan.

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