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The effect of immunoglobulin treatment for hemolysis on the incidence of necrotizing enterocolitis – a meta-analysis

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Abstract. – OBJECTIVE: The application of intravenous immune globulin (IVIG) has been recommended for treating hemolysis in neonates for several years. But in clinical work, more than one study reported that IVIG treatment maybe increased the risk of NEC in hemolytic patients. In light of this situation, we performed this meta-analysis.

MATERIALS AND METHODS: We searched in PubMed, Embase, Cochrane databases for English references, and in Wanfang, VIP, Cnki databases for Chinese references (all last launched on 2015/12/18). Ultimately, 5 studies (Including 4 Chinese articles) were incorporated into this meta-analysis. Odds ratio (OR) and weighted mean difference (WMD) were calculated using a random-effects or fixed-effects model, depending on the data type and heterogeneity of the included studies.

RESULTS: (1) Baseline data including gestational age, gender and TBil between IVIG and control groups were compared in hemolytic infants, and showed no significance. (2) With respect to possible inducement of NEC, SGA and formula feeding were found no significance between IVIG and control groups. In contrast, birth weight was found significantly different between the two groups (WMD = 33.35; 95% CI, 20.70-46.01; $p < 0.00001$). (3) Regarding the incidence of NEC and mortality, the result showed that there was a significant difference between the IVIG and the control groups in the risk of NEC (OR: 4.53; 95% CI, 2.34-8.79; $p < 0.00001$).

CONCLUSIONS: Our results indicate that IVIG treatment for hemolysis may increase the risk of NEC in infants. But it does not increase the risk of final mortality.

Key Words:
Necrotizing enterocolitis, IVIG, Meta-analysis, Hemolysis.

Introduction

As an acute inflammatory necrosis of the intestinal tract, necrotizing enterocolitis (NEC) is the most common acquired gastrointestinal disease for infants in neonatal intensive care units (NICU). NEC has also been a leading cause of morbidity and mortality in infants. According to some annual statistics for the United States, approximately 20-30% of diagnosed NEC patients among very low birth weight (VLBW) infants will die as a result of this disease and its complications[1]. A similarly high annual mortality, approximately10-50%, occurs in China for preterm infants with NEC[2].

Among the numerous theories for pathogenesis, there is wide agreement that NEC is a complicated syndrome characterized by intestinal injury, inflammation, and necrosis. It is characterized by a diversity of alterations in mucosal defenses, gastrointestinal microbiota, and imbalances of inflammatory responses, thus implicating a multi-factorial pathophysiology-including host factors, enteral feeding, abnormal bacterial colonization, and inflammatory propensity of the immature gut[3-5].

The application of intravenous immune globulin (IVIG) has been recommended for treating hemolysis in neonates for several years. But in clinical work, more than one study reported that IVIG treatment maybe increased the risk of NEC in hemolytic patients[6,7]. For example, Kara et al[6] once reported that a female baby developed NEC after two courses of 1 g/kg of IVIG infusion for treating ABO immune haemolytic disease. However, due to lack of support from evidence-based medicine and animal experiments, these doubts still remained to date.

In light of this situation, our meta-analysis was designed for the first time to attempt to clarify the uncertainties in three areas. First was the comparison of baseline data of IVIG treatment on NEC in hemolytic infants. Second, with re-

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spect to the possible inducement of NEC, the SGA, birth weight and formula feeding were respectively compared between the IVIG and control groups. Third, the potential effect of IVIG treatment on the incidences of NEC and death were finally explored.

Materials and Methods

Study Selection

Guidelines from the CONSORT (CONsolidated Standards Of Reporting Trials) group and the CONSORT statement were followed for this systematic review and meta-analysis[8,9]. In order to screen eligible studies published since each database was established, a search was conducted by two investigators involved in this research in PubMed, Embase, and Cochrane databases for studies in English, and in Wanfang, VIP, and Cnki databases for Chinese studies (databases were last launched on 2015/12/18). The following search terms were employed: "necrotizing enterocolitis," "immunoglobulin," "IVIG," "NEC," "globulin," and "hemolysis." The inclusion criteria of this meta-analysis were as follows: (1) controlled test involving hemolytic infants with IVIG treatment; (2) reporting the incidence of NEC (according to the modified Bell staging criteria[10]). Hence, cases, reviews, meta-analyses, animal experiments, and studies without sufficient clinically relevant data were excluded. Any discrepancies were independently resolved by a third investigator involved in this research.

Data Abstraction

The CONSORT statement contains 22 items including participants, intervention, objectives, outcomes, randomization, blinding, statistical method, participant description, recruitment, baseline data, and others. The quality of all included studies was assessed by the CONSORT items and Jadad score. Finally, from the full-text and corresponding supplement information, the following eligibility items were collected and shown in tables for each study: author, year of publication, participants, birth weight, gestation, IVIG dose, baseline data, formula feeding, age, gender, TBil, follow-up, randomization, blinding, Jadad score, and CONSORT items. Subsequently, the outcomes were divided into three parts. First was the comparison of baseline data of IVIG treatment on NEC in hemolytic infants. Second, with respect to the possible inducement of NEC, the SGA, birthweight and formula feeding were compared between IVIG and control groups. Third, the potential effect of IVIG on NEC and death were further explored.

Statistical Analysis

For each outcome, either odds ratio (OR) or weighted mean difference (WMD) with the 95% confidence interval (95% CI) was calculated, depending on the data type. Both a fixed-effects model and a random-effects model were considered. For each meta-analysis, the χ^2-based Q statistic test (Cochran Q statistic)[16] was applied to test for heterogeneity; the I^2 statistic was also used to quantify the proportion of the total variation attributable to heterogeneity[17]. For p-values < 0.10 or $I^2 > 50$, the assumption of homogeneity was assumed to be invalid, and the random-effects model was used; for p-value ≥ 0.10 and $I^2 \leq 50$, data were assessed using the fixed-effects model. Publication bias was investigated by funnel plot, and an asymmetric plot suggested possible publication bias. Statistical analyses were performed using Review Manager 4.2 (Cochrane Collaboration, Nordic Cochrane Centre). A two-tailed p-value of less than 0.05 was deemed statistically significant.

Results

Demographic Characteristics of the Studies

After searching the above databases, 44 potentially relevant studies were obtained. Details of the searching process are shown in Figure 1. A search of other aforementioned databases did not identify any additional eligible studies. Ultimately, we identified 5 original studies (1 in English, 4 in Chinese), including the IVIG group (n=521) and the control group (n=834) (Table I). The quality of all studies included in this meta-analysis was assessed by Jadad score and CONSORT items (Table II).

The Comparison of Baseline Data Between IVIG and Control Groups in Hemolytic Infants

1. With respect to gestational age, data on definite NEC were reported by 3 trials (IVIG group/control group = 304/459) (Figure 2). There was no significant heterogeneity among these trials ($\chi^2 = 3.20$, $p = 0.20$; $I^2 = 37.5\%$). Meta-analysis of data showed no significant difference between IVIG/control groups (95% CI, -0.10-0.27; $p = 0.37$).

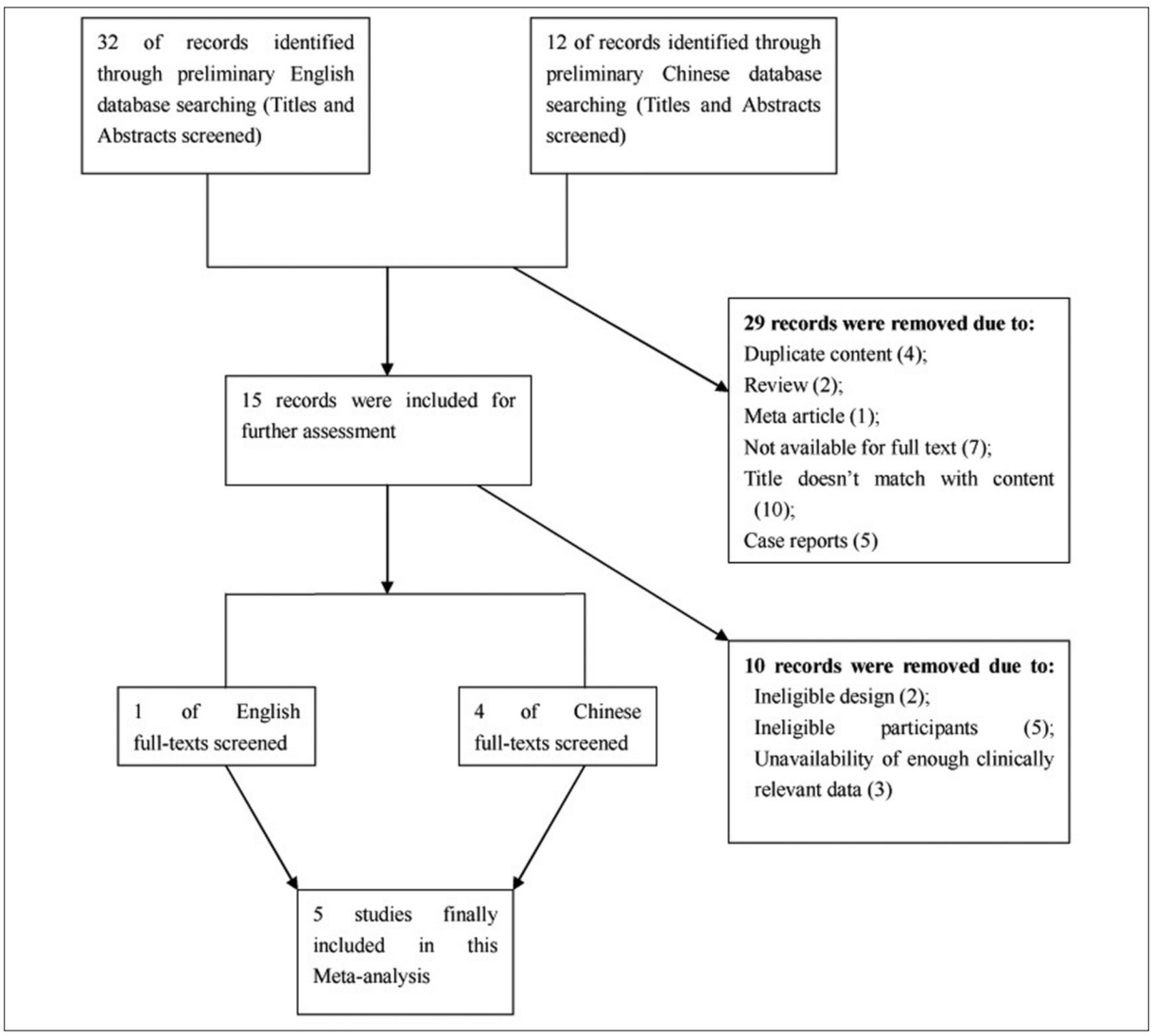

Figure 1. Flow diagram of selection of studies for inclusion in the meta-analysis.

2. Regarding the ratio of gender, there were 4 eligible studies included (IVIG group/control group = 471/784), and no significant heterogeneity was detected among these trials (χ^2 = 1.30, p = 0.73; I^2 = 0%). No significant difference was found in the two groups (95%CI, 0.72-1.16; p = 0.46) (Figure 3).

3. With respect to the level of TBil, 4 studies were included in this meta-analysis (IVIG group/control group = 354/509). There was significant heterogeneity among the trials (χ^2 = 37.51, p < 0.00001; I^2 = 92.0%). Therefore, a random-effects model was applied. The analysis showed that there was no significant difference between IVIG and control groups (95% CI, -7.18-1.96; p = 0.26) (Figure 4).

The comparison of Possible Inducement of NEC Between IVIG and Control Groups

1. Data for SGA between IVIG group and control group were reported by 3 studies (IVIG group/control group = 265/550). There was significant heterogeneity among these trials (χ^2 = 5.28, p = 0.07; I^2 = 62.1%). Therefore, a random-effects model was applied. The result showed no difference for SGA in the two groups (95% CI, 0.32-1.67; p = 0.46) (Figure 5).

2. Data for birth weight comparison were reported in 3 researches (IVIG group/control group = 304/459). There was no significant heterogeneity among the trials (χ^2 = 0.35, p = 0.84; I^2 = 0%). Therefore, a fixed-effects model was

Table I. Demographic characteristics of trials included in the meta-analysis.

Reference	Participants (IVIG/control)	IVIG (dose)	Birthweight (g)	Age (hours)	Gender (man/whole)	GA (weeks)	Formula feeding	TBil (umol/L)
Josep Figueras-Aloy[11] 2009	167/325	500 mg/kg and repeated if necessary	3189 (2773–3460) 3190 (2875–3530)	/ /	77/167 *vs.* 166/325	38.7 (37.0-39.5) *vs.* 39.0 (38.0-40.0)	102/167 *vs.* 227/325	318.1 (266.8-352.3) *vs.* 265.1 (239.4-328.3)
Hu YL12 2012	48/175	500-1000 mg/kg and repeated if necessary	3220.0 ± 40.0/ 3186.0 ± 42.0	60.2 ± 45.0/ 69.6 ± 55.3	22/48 *vs.* 86/175	39.1 ± 1.3 *vs.* 39.0±1.6	26/48 *vs.* vs. 84/175	301.9 ± 98.4 *vs.* 271.5 ± 84.2
Wang L[13] 2012	126/139	500-1000 mg/kg	3239.1 ± 328.6/ 3213.4 ±3 38.4	/	79/126 *vs.* 82/139	38.7±1.0 *vs.* 38.5±1.0	/	329.3 ± 27.3 vs. *vs.* 339.4 ± 15.4
Chen JJ[14] 2013	50/50	1000 mg/kg*3d	/	/	/	/	28/50 *vs.* 43/50	371.6 ± 116.2 *vs.* 295.6 ± 68.8
Wang GL[15] 2014	130/145	800-1000 mg/kg*3d	3116.2 ± 438.5/ 3112.0 ± 441.2	34.1 ± 10.5/ 33.7 ± 11.1	60/130 *vs.* 71/145	38.9 ± 1.5 *vs.* 39.1 ± 1.6	68/130 *vs.* 72/145	385.5 ± 39.4 *vs.* 374.3 ± 38.0

"/" means not reported.

applied. Significant difference was found between the two groups (WMD = 33.35; 95%CI, 20.70-46.01; $p < 0.00001$) (Figure 6).

3. Regarding formula feeding, there were 4 eligible studies included (IVIG group/control group = 477/665), and significant heterogeneity was detected among these trials (χ^2 = 11.75, $p = 0.008$; $I^2 = 74.5\%$). No significantly difference was found in the two groups (95% CI, 0.44-1.24; $p = 0.25$) (Figure 7).

The comparison of Incidences of NEC and Death Between IVIG and Control Groups

1. Regarding the potential effect of IVIG on NEC, the incidence of NEC was respectively compared between the two groups in this meta-analysis. Data were reported in 5 studies (IVIG group/control group = 521/834). There was no heterogeneity among these trials (χ^2 = 6.42, $p = 0.17$; $I^2 = 37.7\%$). Therefore, a fixed-effects model was applied. The result showed that there was a significant difference between the IVIG and the control group (OR: 4.53; 95%CI, 2.34-8.79; $p < 0.00001$) (Figure 8).
2. Data for death in the IVIG and control groups were reported in 5 trials (IVIG group/control group = 521/834). There was no significant heterogeneity among these trials ($\chi^2 = 0.54$, p = 0.46; $I^2 = 0\%$). Therefore, a fixed-effects model was applied. The result showed that there was no significant difference for in the IVIG versus the control group (95% CI, 0.15- 5.13; $p = 0.87$) (Figure 9).

Publication Bias

All trials included in the meta-analysis had Jadad quality scores ≥ 3. A funnel plot was performed to assess the potential publication bias in this meta-analysis. In analyzing the effect of IVIG treatment on NEC, we visually evaluated the symmetry of funnel plot shape and did not find evidence of asymmetry (Figure 10).

Discussion

In the past several years, intravenous immunoglobulin therapy has been commonly used in neonates with isoimmune hemolytic disease and alloimmune thrombocytopenia to reduce the need for exchange transfusion [18,19]. It has been generally considered safe. Cochrane reviews also evaluated the efficacy of IVIG in isoimmuniza-

Table II. Report quality of trials included in the meta-analysis (Only part of the data are shown).

Reference	Title and abstract	Baseline data	Randomization	Blinding	Follow-up	CONSORT Items (22)	Jadad score (5)
Josep Figueras-Aloy[11] 2009	Yes	Yes	Yes	No	No	19	4
Hu YL[12] 2012	Yes	Yes	Yes	No	No	19	4
Wang L[13] 2012	Yes	Yes	No	No	Yes	17	3
Chen JJ[14] 2013	Yes	Yes	Yes	No	No	18	3
Wang GL[15] 2014	Yes	Yes	Yes	No	No	17	3

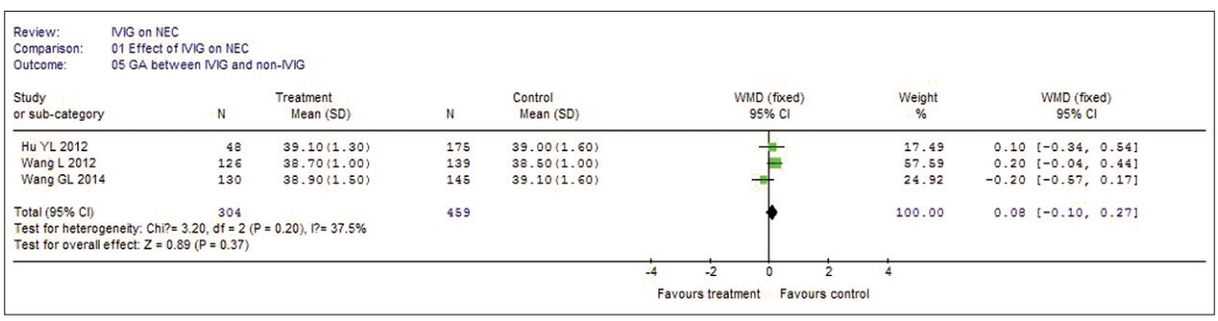

Figure 2. Comparison of gestational age between IVIG and control groups.

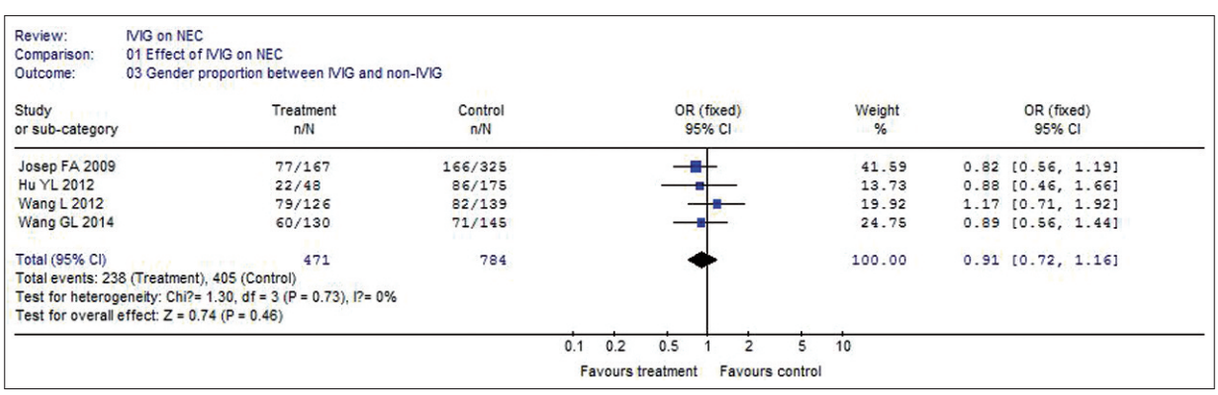

Figure 3. Comparison of gender between IVIG and control groups.

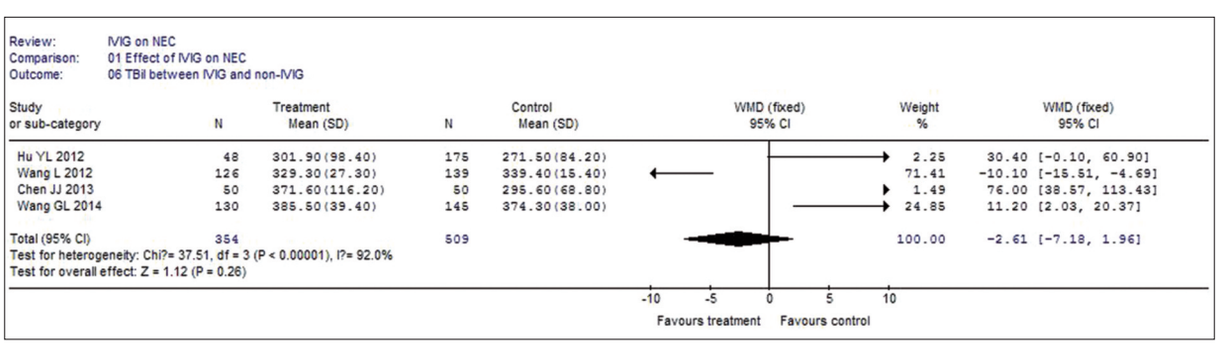

Figure 4. Comparison of TBil between IVIG and control groups.

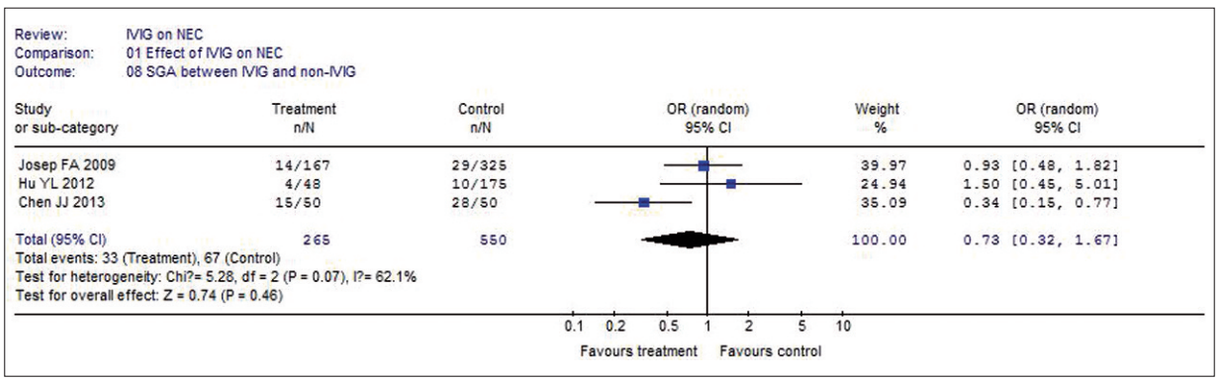

Figure 5. Comparison of SGA between IVIG and control groups.

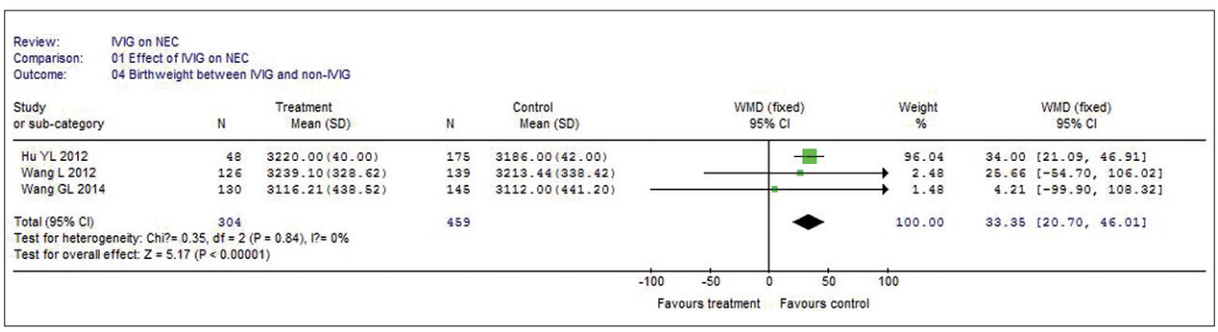

Figure 6. Comparison of birth weight between IVIG and control groups.

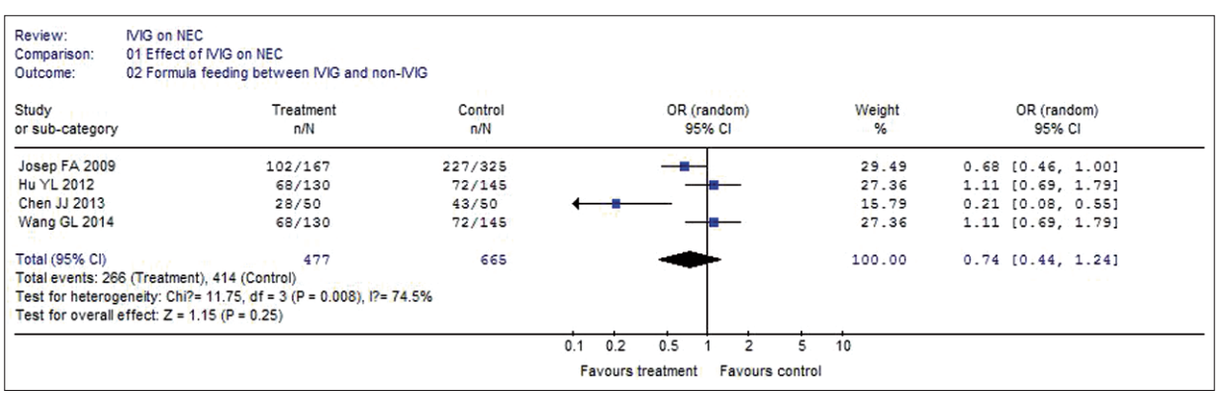

Figure 7. Comparison of formula feeding between IVIG and control groups.

tion and didn't identify any adverse reactions following its administration[18,20]. However, recently an increasing case reports have postulated a possible association between IVIG therapy and NEC in neonates[7,11,21-23].

NEC is one of the most devastating diseases in the NICU, with extremely low birth weight and preterm infants at greatest risk. Data obtained from large, multicenter, neonatal network databases showed a mean prevalence of 7% in infants weighing < 1500 g and an estimated mortality of 15%-30% in the United States and Canada[1,24-26]. Unfortunately, regarding its pathogenesis, NEC has always been viewed as a complex disease, and its etiology has not been clearly elucidated. It appears that multiple factors involving immature in-

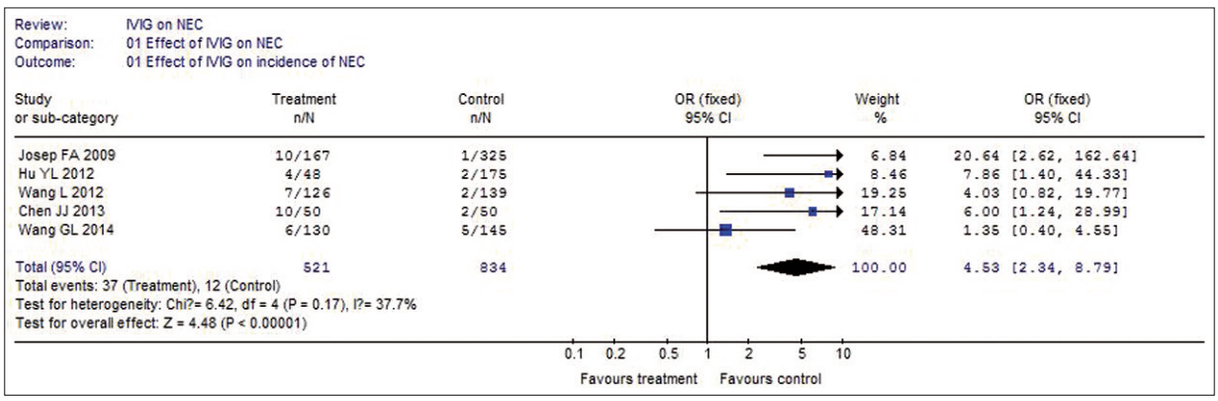

Figure 8. Incidence of NEC between IVIG and control groups.

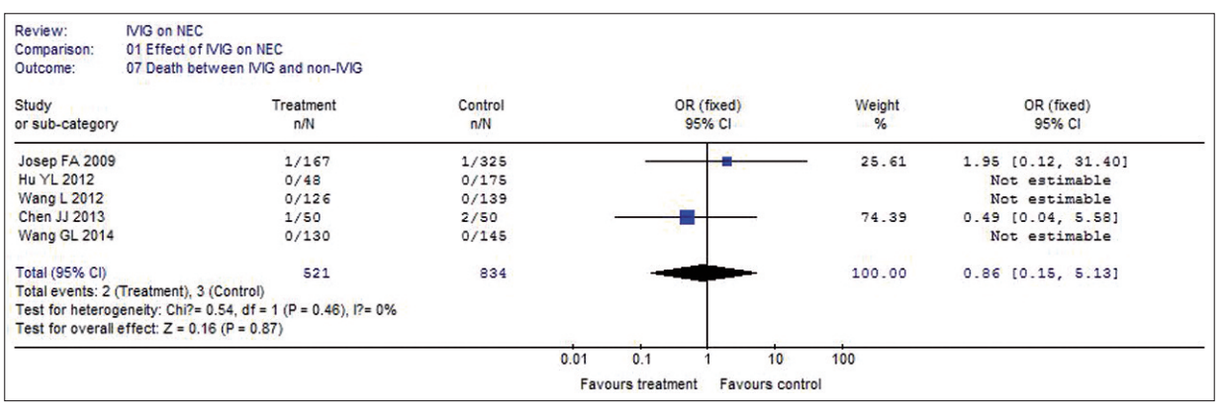

Figure 9. Incidence of death between IVIG and control groups.

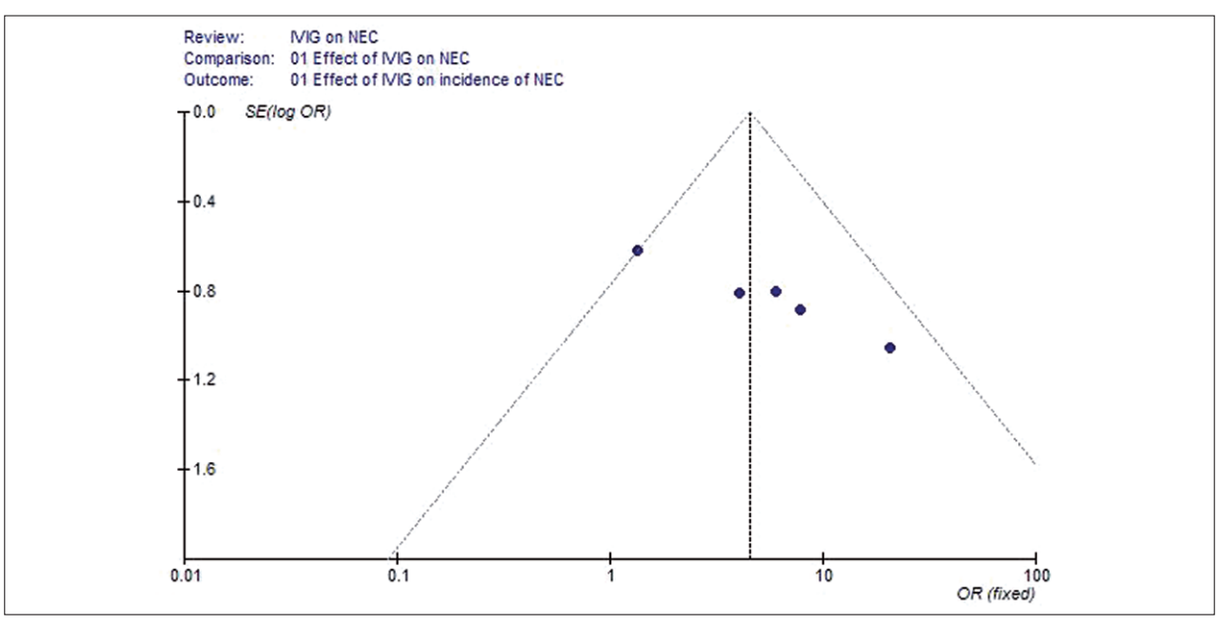

Figure 10. Funnel plot to assess publication bias.

testinal function contribute to the pathogenesis: gastrointestinal dysmotility, impaired digestive capacity, altered regulation of intestinal blood flow, barrier dysfunction, altered anti-inflammatory regulation, and impaired host defenses[27].

Before performing comparisons of the effect of IVIG on NEC, some possible disturbance variables were first analyzed in order to reduce bias. Firstly, the baseline data between IVIG and control groups in hemolytic infants were compared. The results showed that there was no significant difference between IVIG and control groups for gestational age, gender and TBil. (95% CI, -0.10-0.27, $p = 0.37$; 95% CI, 0.72-1.16, $p = 0.46$; 95% CI, -7.18-1.96, $p = 0.26$). Secondly, another factor that needs to be excluded is possible inducement of NEC between IVIG and control groups. We found no significant difference between the two groups in SGA and formula feeding. But with respect to birth weight, significant difference was found between the two groups (WMD = 33.35; 95% CI, 20.70-46.01; $p < 0.00001$). Interestingly, this result seems to have a conflict explanation that infants with greater birth weight form IVIG group are more likely to occurring NEC, which should be more common in low birth weight preterm infants.

Some previous investigations choose to focus on intestinal blood flow change after and 12-18 h following IVIG infusion. The authors hypothesized that intestinal blood flow changes, if at all caused by IVIG, would precede the development of clinical manifestation of NEC. It is theoretically possible that intestinal blood flow changes could occur even beyond this period owing to the long half-life of IVIG. However, they did not find any flow changes even soon after the infusion. There were also no significant changes in superior mesenteric and celiac artery blood flows immediately and 12-18 h after IVIG infusions (1 g/kg) in late preterm and term neonates[28].

IVIG could also increase the platelet counts respectively[28]. Based on this theory, some scholars like Navarro et al[7] and Wittstock et al[29] both observed vein thrombosis after IVIG application. Pathological examination from their experiments revealed bowel resection micro mesenteric vein thrombosis. IVIG could change the expression level of nitric oxide synthase, induced the release of interleukin, TNF-α and endothelin-1, which just induce the contraction of blood vessels. Blood flow change to improve blood viscosity can cause mesenteric ischemia, bowel dilatation, intestinal necrosis, intestinal bacterial overgrowth and translocation, further causing the occurrence of NEC[31].

Another important aspect is whether more severe hemolysis might predispose to intestinal compromise, such that NEC may be associated with hemolysis rather than with IVIG therapy. In Josep Figueras-Aloy's study[11], no statistically significant differences in total serum bilirubin levels according to the presence or absence of NEC were observed. This finding suggests that NEC was unrelated to the severity of hemolysis. Our meta-analysis also showed that though the level of TBil differ between different researches, it has no difference between IVIG and control groups.

In addition to the aforementioned concerns, we must note additional limitations to some recent research. For example, the available studies were almost retrospective studies. In addition, methods of specific randomization and detailed blinding are generally not included in published reports. Some studies include the declaration that the research to date is not adequate to draw precise conclusions. Given these limitations, perhaps the focus of future studies should rather explore in better designed, perspective controlled studies.

Conclusions

Our data indicate that IVIG treatment for hemolysis may increase the risk of NEC in infants. But, our meta-analysis suggests that IVIG does not increase the risk of final mortality.

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Conflict of Interest Statement

None of the authors has a financial relationship with any commercial entity having a potential interest in the subject of this manuscript.

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