

Risk factors associated with poor response to immunosuppressive therapy in acquired aplastic anemia: A meta-analysis of retrospective studies

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Abstract. Acquired aplastic anemia (AA) is a rare hematological disease characterized by bone marrow hypocellularity and varying degrees of pancytopenia. Immunosuppressive therapy (IST) is currently one of the first-line treatments for AA; however, unresponsiveness remains a major concern. Although previous studies have suggested several common risk factors for unresponsiveness, there are currently no widely accepted predictors. Therefore, a meta-analysis of clinical trials including information on factors associated with unresponsiveness of AA to IST was performed in the present study. The PubMed, Embase and Cochrane Library databases were searched for clinical studies on AA evaluating the association between risk factors and unresponsiveness to IST. After the factors were defined from the selected studies, the association between these factors and unresponsiveness to IST was analyzed using Review Manager software. A total of 10 studies comprising 1,820 cases were included in the present meta-analysis. The following factors were identified as predictors of unresponsiveness: Age (≥ 60 years), sex, absolute neutrophil count, severity of the disease, paroxysmal nocturnal hemoglobinuria clone, human leukocyte antigen (HLA)-DR2 and cytogenetic abnormalities (CAs). Among

these factors, only age (≥ 60 years) [odds ratio (OR)=1.65], HLA-DR2 negativity (OR=2.72) and CAs (OR=1.93) exhibited a statistically significant association with unresponsiveness to IST (P=0.006, P=0.04 and P=0.01, respectively). In conclusion, the present meta-analysis revealed that age ≥ 60 years, HLA-DR2 negativity and CAs are risk factors for unresponsiveness to IST. This result may enable clinicians to select an effective therapeutic scheme for patients with AA and even provide novel clues to the pathogenesis of AA.

Introduction

Acquired aplastic anemia (AA) is a rare hematological disease characterized by bone marrow hypocellularity and varying degrees of pancytopenia (1). Although etiological studies have demonstrated that drugs (e.g. chloramphenicol), toxic chemicals (e.g. benzene, pesticides), viruses (e.g. hepatitis virus), autoimmune diseases (e.g. lupus erythematosus) and even pregnancy are associated with AA, the pathogenesis of this disease remains largely elusive (1). The final common sign of bone marrow failure is fatty replacement and a marked decrease in the number of marrow CD34⁺ cells (1); however, the underlying mechanism has remained elusive. In the majority of cases, autoreactive cytotoxic T-lymphocytes cause this disease by suppressing or destroying marrow CD34⁺ cells. However, the shorter telomeres of patients with AA and the approximately three-fold increase in the incidence of AA in certain parts of China indicate that other factors may also contribute to the pathogenesis of AA (1,2).

Although AA is non-malignant, it is a life-threatening condition. Prior to the development of allogeneic hematopoietic stem cell transplantation (allo-HSCT) and immunosuppressive therapy (IST), half of the patients with AA did not survive for >4-6 months (3). Allo-HSCT is generally considered as the first-line treatment for young patients who may have a human leukocyte antigen (HLA)-identical sibling donor (1,4,5). Compared with allo-HSCT, IST is used more frequently, particularly for older patients, and in countries and areas where optimal donors may not be available. Unfortunately, 30-40% of patients with AA respond poorly to IST, although

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the IST regimen has become the standard of care (6). To the best of our knowledge, universally accepted predictors of unresponsiveness to IST have yet to be identified. Therefore, the present meta-analysis was performed to assess several potential predictors of IST unresponsiveness in patients with AA. The results may be helpful in establishing an appropriate therapeutic plan, improving IST efficacy and even providing novel insight into the pathogenesis of AA.

Materials and methods

Data sources and searches. The PubMed (January 1980 to December 2017), EMBASE (January 1980 to December 2017) and Cochrane (January 1980 to December 2017) databases were searched by utilizing the following search terms: ('Immunosuppressive therapy' or 'immunosuppressive treatment') and ('aplastic anemia', 'aplastic anemia' or 'bone marrow failure'). The references of all studies and reviews retrieved were manually scanned to identify additional studies. The language was limited to English.

Study selection. The studies were first screened based on the title and abstract. The full text was then retrieved for further assessment by two authors (WJ and SP). Eligible studies were independently selected by two authors (WJ and SP) according to the following inclusion criteria: a) Clinical trials, prospective studies or retrospective case-control studies; b) diagnostic criteria for severity of AA as determined by the Camitta criteria (Supplemental Table SII) (7); c) anti-thymocyte globulin (ATG) and cyclosporine A (CsA)-based IST regimens; d) response to IST determined using the Bacigalupo criteria (Supplemental Table SIII) (8) and evaluation after a six-month treatment; e) available odds ratios (ORs) and 95% confidence intervals (CIs) of factors affecting no response to IST; f) reported in English.

Data extraction and quality assessment. Two investigators (WJ and SP) independently extracted the data from all eligible studies. Any disagreements were resolved through discussion with senior investigators (JW and XW). The author name, year of publication, journal and country, as well as the number, age and severity of the cases were recorded. Factors associated with unresponsiveness were retrieved from all eligible studies (Table I). The authors were contacted to request any missing information when necessary.

All eligible studies were independently assessed by two investigators (WJ and SP) according to the Newcastle-Ottawa Scale (NOS) (9) (Supplemental Table SI). The NOS scores ranged between 0 (worst) and 9 (best). Scores of 6 to 9 were rated as being of 'high quality', whereas scores of 0-5 were considered to be of 'poor quality'. Disagreements were resolved as described in the data extraction section.

Definition of assessment factors. The factors were selected for assessment according to the following criteria: a) Available ORs of factors affecting no response to IST and 95% CI; b) at least two studies including those data.

Data synthesis and analysis. Data were analyzed using Review Manager software (version for Windows; the Cochrane Collaboration). The heterogeneity of included studies was

assessed using the χ^2 -test ($P < 0.10$) and calculation of the I^2 statistic. The fixed-effects model was applied, but when statistically significant heterogeneity was detected ($P < 0.10$ or $I^2 > 50\%$), the random-effects model was adopted. The results were expressed as ORs with 95% CI and were presented in forest plots. ORs were used to assess the extent of the association of the factors with poor response of the patients with AA to IST. Differences were considered statistically significant if $P \leq 0.05$ (two-tailed). Publication bias was assessed using Begg's funnel plots.

Results

Studies and factors included. Following the search strategy, 3,499 studies were identified, 10 of which were ultimately included in the present meta-analysis (Fig. 1). The characteristics of the studies (10-19) included are summarized in Table I. All of the studies were retrospective and their publication date range was between 1997 and 2011; a total of 1,820 cases in various countries and involving multiple ethnicities were reported, most of which were idiopathic. The mean NOS score was 6.9 (range, 6-7, Table SI), indicating that the quality of the meta-analysis was acceptable. Although the present meta-analysis included the full spectrum of AA (Table I), 4 studies including 469 patients only reported on severe AAb (12,13,16,18) and 2 including 316 patients did not include any data on the severity distribution of the cases (10,11). With regard to the age range of the patients in these studies (10-19, Table I), 5 studies including 795 patients focused on mixed-age groups (10,14,15,17,18), 1 including 316 patients on adult patients (≥ 18 years) (16), 2 including 329 patients on pediatric patients (< 18 years) (13,19), 1 of 99 patients on mixed-age or adult cases (12) and 1 study of 281 patients did not provide the age of the subjects (11).

In the studies included, 7 factors were identified for analysis: Age (≥ 60 years), sex, absolute neutrophil count (ANC), severity of the disease, paroxysmal nocturnal hemoglobinuria (PNH) clone, HLA-DR2 and cytogenetic abnormalities (CAs) (Table I). Although these factors were not included in each study, it was possible to analyze them by combining the data of at least 2 studies from which the ORs were able to be calculated. As a higher age was indicated to be a factor predicting unresponsiveness to IST in previous studies and there is a peak in incidence between the ages of 65 and 69 years (16,20,21), age (≥ 60 years) was included as a factor.

Meta-analysis results for factors included

Age (≥ 60 years). Two studies (16,17) including 640 patients were reported. Due to the lack of significant heterogeneity among the included studies ($P = 0.74$, $I^2 = 0\%$), the fixed-effects model was selected. The total OR (95% CI) was 1.65 (1.16, 2.38). The results of the meta-analysis indicated a statistically significant association between age (≥ 60 years) and unresponsiveness to IST ($P = 0.006$; Fig. 2).

Sex. Four studies (10,15,17,19) including 696 patients were reported. Due to the significant heterogeneity among the included studies ($P = 0.04$, $I^2 = 64\%$), the random-effects model was selected. The total OR (95% CI) was 0.97 (0.48, 1.98). The results of the meta-analysis did not indicate any statistically significant association between sex and unresponsiveness to IST ($P = 0.94$; Fig. 3).

Table I. Characteristics of the studies included.

First author (year)	Journal	Cases (n)	Median age (range)	Country	Severity of AA, n (%)	Factors	QS (Refs.)
Ihan, <i>et al</i> (1997)	Int J Hematol	35	23 (16~57y)	Turkey	Not provided	Age, sex, HLA-DR2	6 (10)
Maciejewski <i>et al</i> (2001)	Blood	281	Not provided	USA	Not provided	HLA-DR2	7 (11)
Sauntharajah <i>et al</i> (2002)	Blood	99	34y, 21±0.7y ^a	USA	SAA, 99 (100)	HLA-DR2	7 (12)
Oguz <i>et al</i> (2002)	Haematologica	17	Pediatric cases	Turkey	SAA, 17 (100)	HLA-DR2	7 (13)
Gupta <i>et al</i> (2006)	Br J Haematol	81	5-73y	UK	NSAA, 39 (48.1); SAA, 28 (34.6); VSAA, 16 (19.8)	CAs	7 (14)
Saracco <i>et al</i> (2008)	Br J Haematol	42	15-239m	Italy	NSAA, 6 (14.5); SAA, 19 (45.0); VSAA, 17 (40.5)	Age, sex, ANC, platelet, HLA-DR2, severity, interval from diagnosis to therapy	7 (15)
Scheinberg <i>et al</i> (2009)	Br J Haematol	316	31y (18-52y)	USA	SAA, 316 (100)	Age, ANC, ARC, ALC, PNH clone	7 (16)
Kim <i>et al</i> (2010)	Genes Chromosomes Cancer	600	67y (17-87y)	Korea	NSAA, 301 (50.2); SAA, 255 (42.5); VSAA, 44 (7.3)	Age, sex, severity, PNH clone, CAs, HB(C)V	7 (17)
Song <i>et al</i> (2010)	Hum Immunol	37	35y (3-66y)	Korea	SAA, 37 (100)	HLA-DR2	7 (18)
Yoshida <i>et al</i> (2011)	Haematologica	312	8y (<18y)	Japan	NSAA, 49 (15.7); SAA, 107 (34.3); VSAA, 156 (40.4)	sex, severity, etiology, peripheral blood parameters at diagnosis, interval from diagnosis to therapy	7 (19)

^aMean age in two different groups; QS, quality assessment; y, years; m, months; NSAA, non-severe aplastic anemia; VSAA, very severe aplastic anemia; ANC, absolute neutrophil count; ARC, absolute reticulocyte count; ALC, absolute lymphocyte count; PNH, paroxysmal nocturnal hemoglobinuria; HB(C)V, hepatitis B (C) virus.

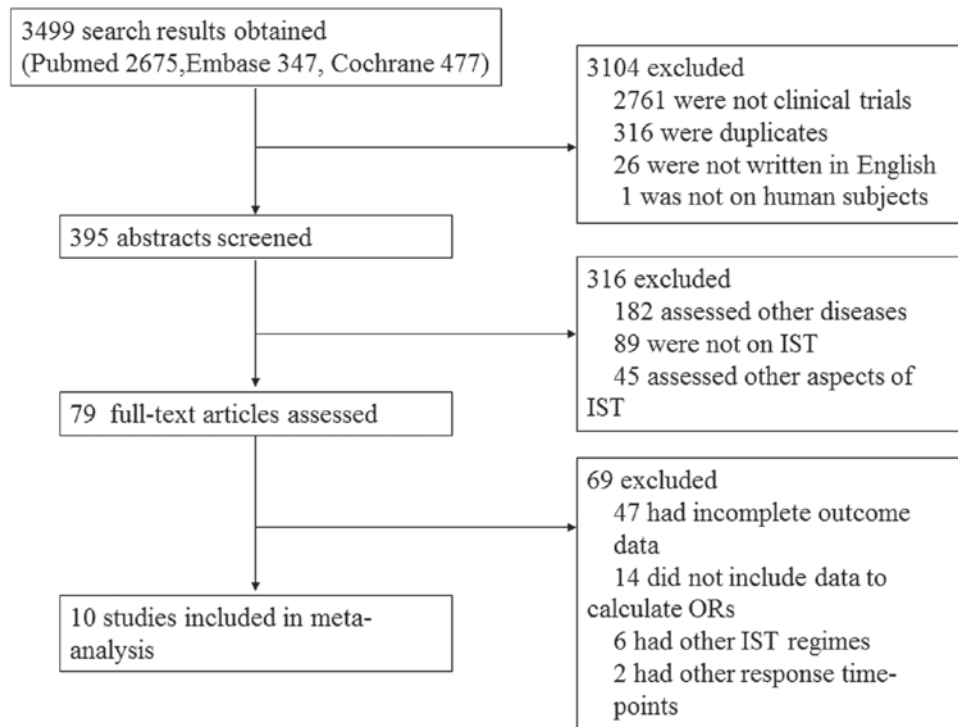


Figure 1. Flow chart depicting the selection process of studies for the present meta-analysis. OR, odds ratio; IST, immunosuppressive therapy.

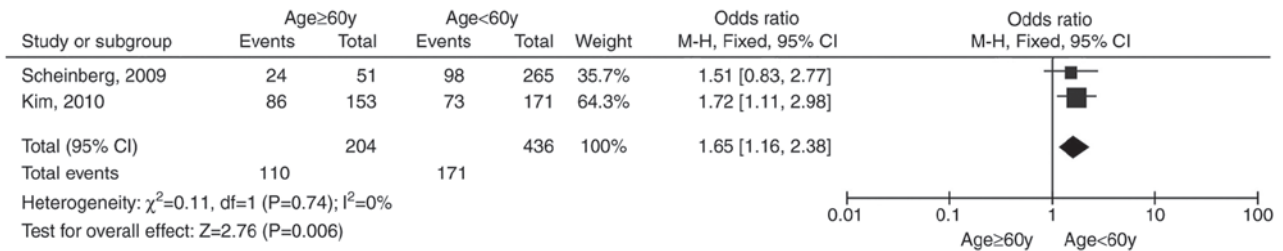


Figure 2. Forest plots of poor response to immunosuppressive therapy according to age. M-H, Mantel-Haentzel; df, degrees of freedom; y, years.

ANC. Two studies (15,16) including 354 patients were reported. Due to the significant heterogeneity among the included studies ($P=0.02$, $I^2=81\%$), the random-effects model was selected. The total OR (95% CI) was 0.67 (0.05, 8.74). The results of the meta-analysis did not indicate a statistically significant association between ANC and unresponsiveness to IST ($P=0.76$; Fig. 4).

Severity of disease. Two studies (17,19) including 636 patients were reported. Due to the significant heterogeneity among the included studies ($P=0.09$, $I^2=65\%$), the random-effects model was selected. The total OR (95% CI) was 1.00 (0.53, 1.87). The results of the meta-analysis did not indicate any statistically significant association between the severity of the disease and unresponsiveness to IST ($P=0.99$; Fig. 5).

PNH clone. Two studies (16,17) including 444 patients were reported. Due to the significant heterogeneity among the included studies ($P=0.007$, $I^2=86\%$), the random-effects model was selected. The total OR (95% CI) was 2.85 (0.31, 25.77). The results of the meta-analysis did not indicate any statistically

significant association between PNH clone and unresponsiveness to IST ($P=0.35$; Fig. 6).

HLA-DR2. 6 studies (10-13,15,18) including 486 patients were reported. Due to the significant heterogeneity among the included studies ($P=0.006$, $I^2=70\%$), the random-effects model was selected. The total OR (95% CI) was 2.72 (1.06, 7.00). The results of the meta-analysis indicated a statistically significant association between HLA-DR2 negative status and unresponsiveness to IST ($P=0.04$; Fig. 7).

CAs. Two studies (14,17) including 405 patients were reported. Due to the lack of significant heterogeneity among the included studies ($P=0.52$, $I^2=0\%$), the fixed-effects model was selected. The total OR (95% CI) was 2.93 (1.24, 6.95). The results of the meta-analysis indicated a statistically significant association between CAs and unresponsiveness to IST ($P=0.01$; Fig. 8).

Publication bias. As indicated by the funnel plots (Fig. 9), the studies providing age (≥ 60 years), disease severity and CAs analysis were inside the 95% CIs, with an even distribution

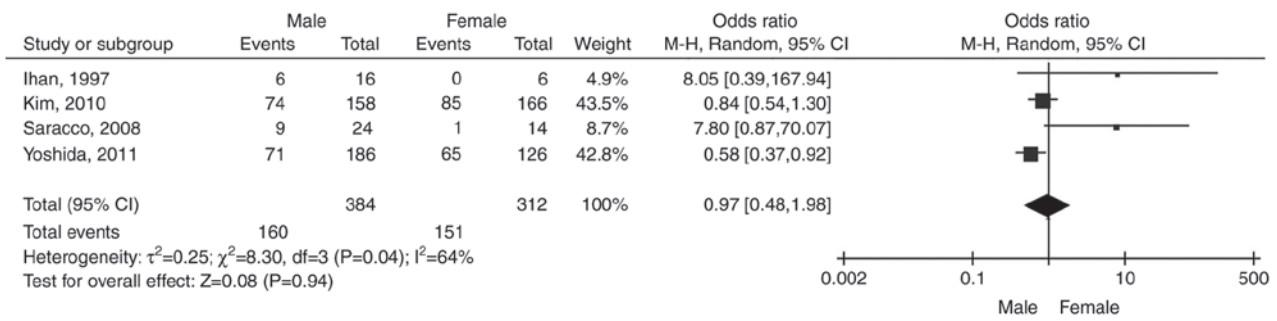


Figure 3. Forest plots of poor response to immunosuppressive therapy according to gender. M-H, Mantel-Haentzel; df, degrees of freedom.

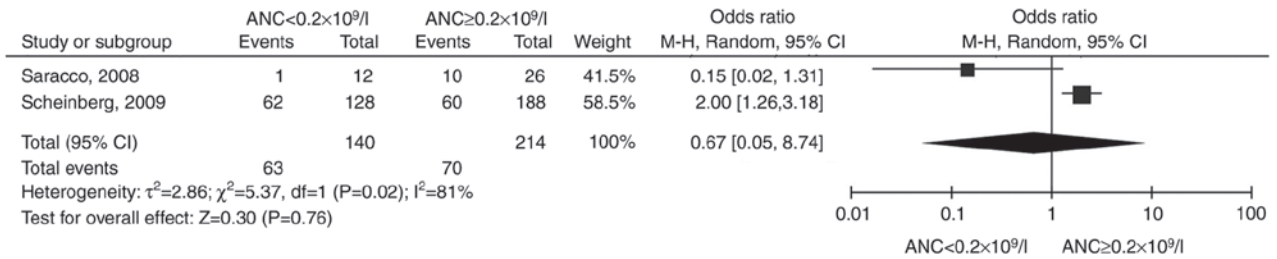


Figure 4. Forest plots of poor response to immunosuppressive therapy according to ANC. M-H, Mantel-Haentzel; df, degrees of freedom; ANC, absolute neutrophil count.

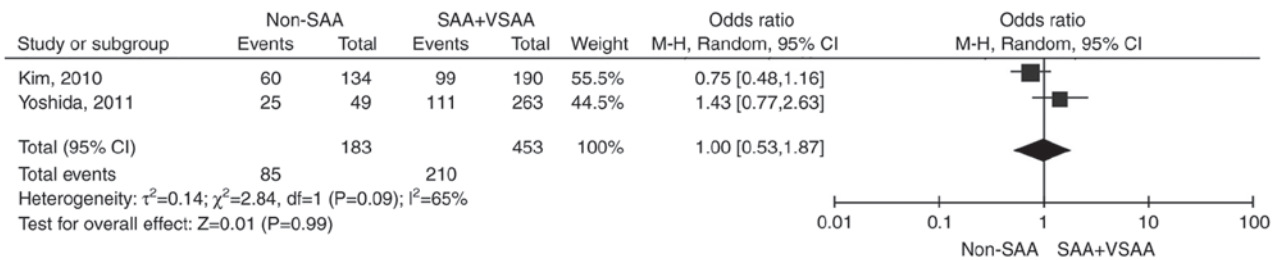


Figure 5. Forest plots of poor response to immunosuppressive therapy according to the severity of the disease. M-H, Mantel-Haentzel; df, degrees of freedom; VSAA, very severe aplastic anemia.

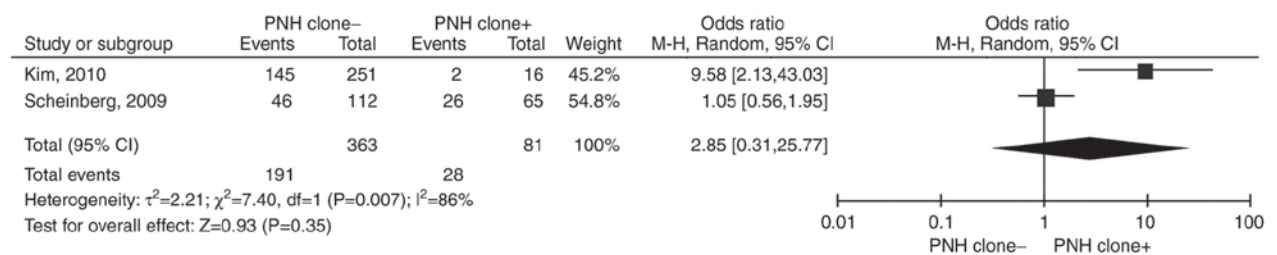


Figure 6. Forest plots of poor response to immunosuppressive therapy according to PNH clone. M-H, Mantel-Haentzel; df, degrees of freedom; PNH, paroxysmal nocturnal hemoglobinuria.

around the vertical symmetry, which indicates that there was no publication bias. However, the other factors were associated with publication bias, with asymmetrical funnel plots (Fig. S1).

Discussion

AA is a clinical manifestation of bone marrow failure, wherein the hematopoietic stem and precursor cells are nearly absent and are replaced by fat. Accordingly, the varying degrees

of decrease in the number of peripheral blood cells result in anemia, infection and bleeding (1,22). HSCT and IST have become the first-line treatments for AA and the 5-year survival rates are similar for patients receiving those therapies (1,22). However, allo-HSCT is limited by age, requirement for a suitably matched sibling donor and post-transplantation complications. Thus, IST is the modality most widely used in AA patients in several countries and geographical areas (1,22). Although IST is one of the most effective treatments for AA,

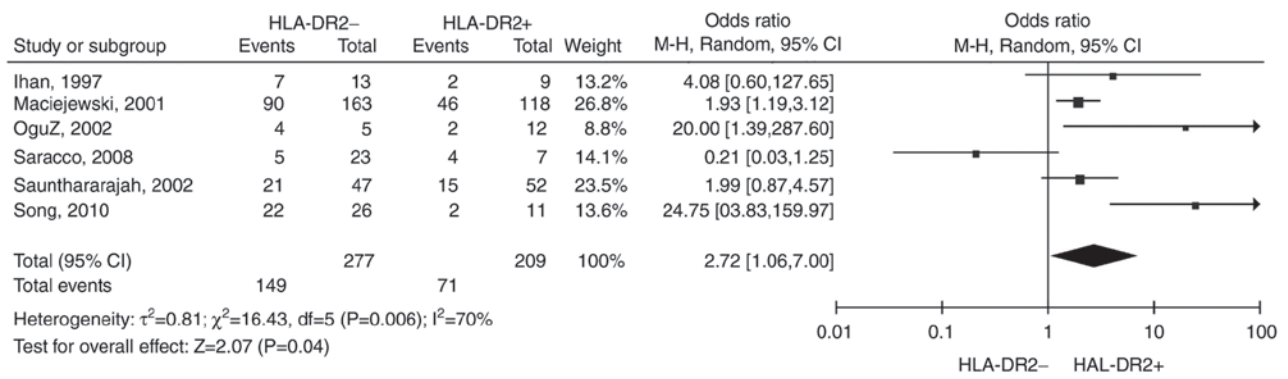


Figure 7. Forest plots of poor response to immunosuppressive therapy according to HLA-DR2. M-H, Mantel-Haentzel; df, degrees of freedom; HLA, human leukocyte antigen.

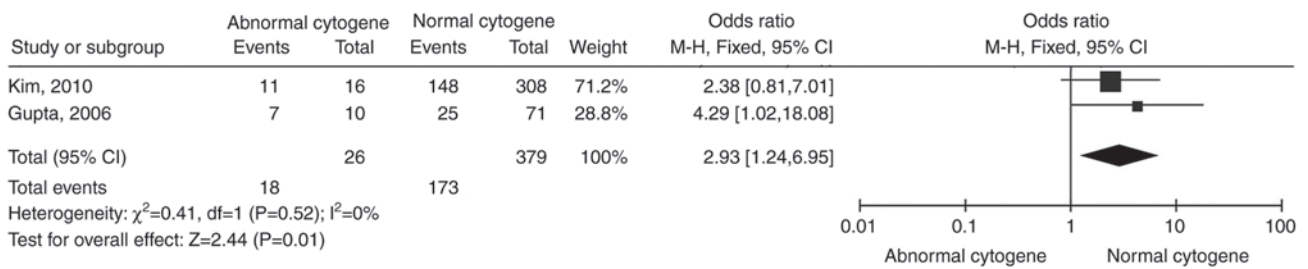


Figure 8. Forest plots of poor response to immunosuppressive therapy according to CAs. M-H, Mantel-Haentzel; df, degrees of freedom; CA, cytogenetic abnormalities.

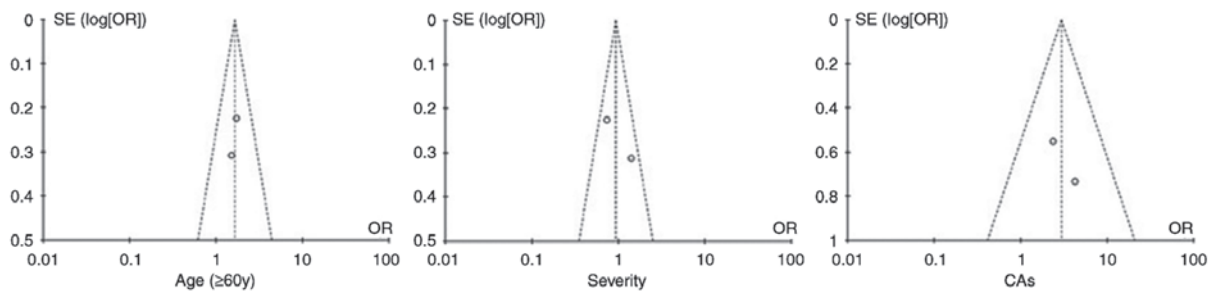


Figure 9. Funnel plots for the assessment of publication bias for the factors of age, severity and CAs. SE, standard error; OR, odds ratio; CAs, cytogenetic abnormalities; y, years.

it is associated with several problems, including unresponsiveness, relapse and malignant transformation (1,22). Several common risk factors associated with unresponsiveness have been reported by previous studies in different populations and areas (1,10-19,22). However, widely accepted predictors of AA have yet to be identified. To the best of our knowledge, the present study was the first meta-analysis of risk factors associated with unresponsiveness to IST in AA. A total of 3 factors, namely age, HLA-DR2 negativity and CAs, were revealed to be statistically significantly associated with unresponsiveness of AA to IST, whereas sex, ANC, disease severity and PNH clone did not exhibit such an association.

Among the three risk factors, old age is most widely recognized. Several studies from different countries and areas have reported that old age is a major prognostic indicator of poor response to IST in AA (20,23). Age is also associated with the incidence of AA (1), with two incidence peaks

observed between 15 and 25 years, as well as between 65 and 69 years (1). However, old age is usually negatively associated with the incidence of autoimmune diseases (21,24). In addition, anemia in old patients has the unique characteristics of clinical presentation and prognosis (25,26). These results suggest that anemia in old patients may have a distinct pathogenesis. Certain studies consider that the high incidence and specific presentation of older patients with AA may be linked to telomere length and hematopoietic stem cell number (2,4). However, these previous studies lack convincing evidence and even contradict one another. For instance, numerous studies pointed out that short telomere length may favor the development of AA (2,4,27,28), but older patients with AA do not have a shorter telomere length compared with healthy older subjects (26). Therefore, old patients with AA should be more thoroughly investigated, particularly regarding the pathogenesis of this disease, in order to improve its treatment.

Similar to age, HLA-DR2 was indicated to be associated with response to IST in previous studies (10,11,13,15,18). In general, patients with HLA-DR2 exhibit a good response to CsA or the combined protocol of CsA + ATG/ALG, but not to ATG/ALG alone. The mechanism underlying their association remains elusive, but the fact that HLA-DR2 is highly expressed in patients with AA and is associated with a good response to IST suggests a key role for HLA-DR2 in AA. It was previously hypothesized that HLA-DR2, which is frequently over-represented in autoimmune diseases, including anti-glomerular basement membrane disease (29) and multiple sclerosis (30), may be a predisposing factor for the immune process of autoimmune diseases, partly as certain HLA haplotypes are not only susceptible to present foreign/self-antigen-derived pathogenic peptides, but are also involved in the formation of autoantigens (13,29,30). In addition, HLA-DR2 is associated with a decrease in the production of tumor necrosis factor (TNF)- α (31,32). However, TNF- α release increases following activation of interferon (IFN)- γ (32). Furthermore, the TNF- α and IFN- γ cytokines have an important role in the depletion of bone marrow CD34⁺ cells in AA (1). Therefore, HLA-DR2 may be involved in the formation of AA through an autoimmune mechanism to a certain extent, whereas HLA-DR2-negative patients with AA may harbor mechanisms other than autoimmunity. This condition is associated with a poor response to IST.

Compared with the former factors, there are certain issues regarding the association between CAs and response to IST in AA. In certain institutions, AA with an abnormal karyotype at the time of diagnosis is considered as myelodysplastic syndrome (33); however, the incidence of CAs at initial diagnosis is approximately 4-15% (17,19,34), which is not accompanied by morphological changes (17,19,34-39). The mechanism and function of CAs in AA have remained to be fully elucidated due to the scarcity of uniform, systematic and large-scale studies. Based on previous studies, trisomy 8, trisomy 6 and monosomy 7 are the most common CAs among patients with AA (17,19,34-39). Trisomy 8 and trisomy 6 are frequently present at the time of diagnosis, whereas monosomy 7 almost always develops after treatment (17,19,34-39). Studies on various ethnic groups and geographical areas report that trisomy 8 responds well to IST (17,19,33,39), whereas the opposite was observed for trisomy 6 and monosomy 7 (17,19,33,36,39), with trisomy 6 reported to be 'the sole predictable marker for IST unresponsiveness' (34). The CAs of AA are not constant over the course of the disease and are transiently present in certain cases (38-40). The different clinical courses of CAs may be associated with their different biology after development, including Fas expression, sensitivity to external stimuli and gene expression profiles, although all of them are considered to initially result from genome instability under immune pressure (41-46). Although the CAs of AA occur at random and are secondary cytogenetic events, the fact that an individual CA is always associated with a specific clinical presentation, along with the multifactorial pathogenesis of AA, cannot exclude the possible involvement of the initial CAs in CD34⁺ cell depletion (41,42). For instance, trisomy 6, one of the most common CAs in AA and a predictor of

poor response to IST, was characterized by hypoplastic bone marrow in most studies and was observed in early hematopoietic progenitor cells (10,46,47), suggesting that trisomy 6 may be an intrinsic factor causing bone marrow hypoplasia. These results suggest different molecular mechanisms of action and different behavior of these CAs in AA; therefore, further studies should assess individual CAs, rather than CAs as a group.

There were several limitations to this meta-analysis. First, the factors including sex, ANC, PNH clone and HLA-DR2 are associated with publication bias and the quality of included studies are only 6-7 out of 10 on NOS score although three databases were searched. Furthermore, all of the studies included are clinical trials, wherein the deficiencies may be magnified through meta-analysis. In addition, in three of the selected studies, although the source of the patients was different, they were from the same area; thus, the same patients may have been included in more than one study, which may overemphasize the results. However, without more details, it is difficult to determine whether this magnification exists and to what extent. Furthermore, the quality of original data from the studies included cannot be improved by the present meta-analysis. In addition, each factor was analyzed using data from different studies. Using the same studies would be ideal for the analysis of these factors; however, due to the scarcity of published studies on response to IST in AA, there were no more than two studies that shared more than two factors. Finally, only univariate analysis was performed in the present meta-analysis, which may compromise the accuracy of the conclusions. This limitation is attributed to the fact that the analysis results were obtained from different sources. However, the intention was not to reach a definitive conclusion, but rather to detect risk factors of AA unresponsiveness to IST for future research.

In conclusion, the present study demonstrated that age (≥ 60 years), HLA-DR2 negativity and CAs are risk factors of unresponsiveness to IST. This result may be helpful for establishing a treatment guideline for AA. Furthermore, this result may even provide novel clues to the pathogenesis of AA. Some studies suggest that in addition to an abnormal immune system, other factors including telomere length, HLA or CAs, may trigger or be involved in the development of this disease (48-50), which may lead to diverse patterns of responsiveness to IST. Important future research includes large, multicenter clinical trials and laboratory research, in order to help fully elucidate the pathogenesis of AA.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

All authors contributed to the scientific work and therefore share collective responsibility and accountability for the results. WJ and XW conceived and designed the study. JW and PS collected and analyzed the data and interpreted the results; JW and PS drafted the manuscript. All authors have read the manuscript and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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