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Multiple risks analysis for aplastic anemia in Zhejiang, China

A case-control study

Li-qiang Wu, MM[a], Ying-ying Shen, MM[a], Ying Zhang, MM[a], Yue-min Kuang, MB[b], Bing-mu Fang, MM[c], Zhou-zheng Wang, MB[d], Li-juan Fu, MB[e], Ke-ding Shao, MM[f], Jian-ping Shen, MB[a], Yu-hong Zhou, MB[a], Yi-ping Shen, MB[a], Bao-dong Ye, MD[a], Zhi-yin Zheng, MD[a], Jun-fa Chen, MM[a], Sheng-yun Lin, MB[a,*]

Abstract

To understand the risks associated with aplastic anemia (AA) in 4 cities of Zhejiang Province, China, with special focus on the joint contributions of multiple risks.

Based on an Electronic Data Capture (EDC), a case control study was carried out. Data regarding socio-demographic, diseases history, living habits, and exposures to toxic substances, etc., were collected through survey questionnaires. *t* Test, chi-square test, or non-parametric rank sum test, and univariate and multivariate Logistic regression analysis were conducted to analyze data.

The univariate logistic regression analysis results indicated that among all study participants (n = 1802), AA was associated with over 30 risks, in terms of their individual behaviors, daily and environmental exposures, diseases history, and family history. Multivariate logistic regression analysis further confirmed that the independent risks related to AA included presence of chemical factory within 3 km of living residence (odds ratio [OR] = 8.73, 95% CI: 1.42–53.74, $P = .019$), living in a newly decorated house/apartment (OR = 25.37, 95% CI: 4.44–144.81, $P < .001$), vegetarian diet (OR = 131.60, 95% CI: 3.45–5020.16, $P = .009$), preference of sugar (OR = 89.38, 95% CI: 7.22–1106.44, $P < .001$), preference of oily food (OR = 55.68, 95% CI: 5.12–605.26, $P = .001$), drinking lake water or pond water (OR = 58.05, 95% CI: 3.21–1049.81, $P < .001$), habit of staying up late (OR = 11.87, 95% CI: 3.43–41.02, $P < .001$), infection history (OR = 10.08, 95% CI: 2.75–36.93, $P < .001$). Result of receiver operating characteristic curve (ROC) analysis on the joint contribution of multiple risks indicated that AA was 13.835 times likely to occur when exposed to ≥1 risks than those exposed to 0 risks (95% CI: 9.995–19.149).

Our study results demonstrated a comprehensive epidemiological pattern, in which the joint contributions of individual inherited health status, environment exposure, and individual behaviors lead to the occurrence of AA.

Abbreviations: AA = aplastic anemia, EDC = electronic data capture, GSTM1 = glutathione S-transferase Mu 1, OR = odds ratio, ROC = receiver operating characteristic curve, SCE = sister chromatid exchange, VOCs = volatile organic compounds.

Keywords: aplastic anemia, case-control study, risk factor

1. Introduction

Aplastic anemia (AA) is a bone marrow hypoplasia syndrome resulting in decrease in white blood cells, hemoglobin, and platelets, and its clinical symptoms include anemia, bleeding, and infection.[1] Severe AA has a high mortality rate. While non-severe AA has slow progress and long course, and some even need transfusion to maintain treatment, which could badly influence patients' quality of life. At present, the main therapies include immune-suppression and allogeneic hematopoietic stem cell transplantation, by which 60% to 80% patients' condition could be improved or cured.[2,3] However, these therapies had adverse effect and were expensive, which increased the burden of their family and the society. The actual cause of AA is still unclear. At present, the known risks causing damage to hematopoietic system of bone marrow include physical, chemical, and biological ones, or their common effects.[4] Therefore, our focus is on identifying potential etiological risks that would help to prevent AA in the future.

Most of epidemiologic surveys on AA were conducted in 1980s in China. A large national survey, which was implemented in 21 provinces of China between 1986 and 1988, reported that the incidence rate of AA was $7.4/10^6$.[5] However, the incidence rate was only $3.7/10^6$ in 1991 in Bangkok,[6] which was obviously lower than that in China. Besides, the incidence in France was

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[a] Department of Hematology, The First Affiliated Hospital of Zhejiang Chinese Medical University, [b] Department of Hematology, Jinhua People's Hospital, Jinhua, [c] Department of Hematology, Lishui City People's Hospital, Lishui, [d] Department of Hematology, The Second Hospital of Jiaxing, Jiaxing, [e] Department of Hematology, The Second Affiliated Hospital of Zhejiang Chinese Medical University, [f] Zhejiang Chinese Medical University, Hangzhou, China.

[*] Correspondence: Sheng-yun Lin, Department of Hematology, The First Affiliated Hospital of Zhejiang Chinese Medical University, 54 Youdian Road, Hangzhou 310006, China (e-mail: linshengyun1113@163.com).

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even lower, that is, only $1.5/10^6$, based on a national survey in 83 medical centers of France between May 1984 and April 1987.[7] This indicated that the incidence rate in Asia may be 2 to 3 times of that in Western countries. The booming industry has worsened environmental pollution, and altered living environment and lifestyle of human beings over years.[8] New cases of AA have trended to increase over years; while risks associated to AA may have also changed over time. In recent years, plenty of studies have been carried out in China regarding AA. However, most of them were clinical case analysis, or focused on one single risk of AA, or had a small study sample. There have been few reports on the association between multiple risks and AA.[8–10]

In addition, the previous study on disease related risks relied mainly on questionnaires and clinical records, which prolonged study analysis for complex data collection. We adopted Electronic Data Capture (EDC) system to collect and manage data, which has shortened data collection and cross-check and improved the quality of data. Researchers could input data simultaneously by using EDC in 5 participating medical centers. At present, EDC has been mainly applied in clinical trials of drugs, but seldom been used in epidemiological study of chronic diseases.

Therefore, based on 10 years' patients' data in EDC from 5 medical centers in 4 cities, we designed this study to further confirm the association between varied risks and AA, and analyze the epidemic features of AA in China, by using hospital-based database. We hope this study could lay the groundwork for assessing AA-related risks and provide evidence for prevention of AA among the high-risk population.

2. Materials and methods

2.1. Study setting and participants

A case–control study was conducted in 4 cities of Zhejiang Province, eastern coastal province of China. The participants included in-hospital patients from 5 major medical centers of Hangzhou, Lishui, Jiaxing, and Jinhua City from January 1st, 2003 to January 1st, 2014. The study had acquired the approval of ethnics committee of the First Affiliated Hospital of Zhejiang Chinese Medical University, with reference number of 2014-KL-018–01. The Clinical Assessment and Analysis Center of the First Affiliated Hospital of Zhejiang Chinese Medical University was responsible for data management and supervision in this study.

2.2. Cases

Cases participants were recruited from the First Affiliated Hospital of Zhejiang Chinese Medical University, the Second Affiliated Hospital of Zhejiang Chinese Medical University at Hangzhou City, Lishui City People's Hospital, the Second Hospital of Jiaxing, and Jinhua People's Hospital. Eligible cases included patients who met the diagnosis criteria of AA (Table 1)[11] and were willing to participate in the study. Those whose medical records were not accessible and who were dead were not included. Exclusion criteria included history of tumor, radiotherapy and chemotherapy, immunological therapy, other blood system diseases, immune disorders such as lupus erythematosus and ankylosing spondylitis, hypersensitivity diseases, and congenital disease such as Fanconi anemia.

2.3. Controls

The controls were selected by matching age, sex, and similar period of hospital admission (±6 months) with cases, in a ratio 4 to 1. They were also inpatients from departments of pediatrics, gastrointestinal surgery, hepatobiliary surgery, orthopedics, encephalopathy, respiration, nephropathy, gastroenterology, cardiovascular. The same exclusion criteria were applied in selecting controls.

Table 1

Diagnosis criteria of aplastic anemia.

Type of AA	Criteria
Severe AA (SAA)	Bone marrow cellularity <25% (or 25–50% with <30% residual hematopoietic cells), plus at least 2 of: neutrophils $< 0.5 \times 10^9$/L. platelets $< 20 \times 10^9$/L. reticulocyte count $< 20 \times 10^9$/L.
Very severe AA (VSAA)	As for SAA but neutrophils $< 0.2 \times 10^9$/L.
Non-severe AA (NSAA)	Bone marrow cellularity <50%, plus at least 2 of: neutrophils $< 1.5 \times 10^9$/L. platelets $< 100 \times 10^9$/L. reticulocyte count $< 40 \times 10^9$/L. >6 weeks. Patients not fulfilling the criteria for SAA or VSAA.

AA = aplastic anemia.

2.4. Data collection tool

The Clinical Assessment and Analysis Centre of the First Affiliated Hospital of Zhejiang Chinese Medical University designed a database based on EDC for this study. Pre-survey was firstly conducted among AA patients in the same Hospital to identify measures and outcomes in this study. The database had limited access to the participating hospitals to ensure confidentiality of all participants.

Data were collected by trained clinical researchers. Informed consents were obtained from the participants before the questionnaire were administered. The questionnaires had 6 modules: general socioeconomic status, including age, sex, education level, medical insurance types, occupation type; daily exposures to chemical factory, ionizing radiation, pesticides; individual behaviors, including smoking, drinking, diet preference, drinking water sources, staying-up late; occupational exposures to industrial toxics; individual health status, including birth status, disease history, pregnancy status for female adults; family disease history (Table 2 for details). The same questionnaire was applied for both case and control groups. Participants provided information themselves unless they had severe condition or were too young to understand the questions, in which cases their family members provided instead. Data were cross-checked and input into EDC. Missing or ambiguous information was re-collected through telephone or email.

2.5. Statistical analysis

We adopted SPSS version 17.0 software (IBM Corp., Armonk, NY) to analyze the collected data statistically. The continuous variables were presented by means ± standard deviation, and the categorical variables were presented in percentage. *t* test, chi-square test or non-parametric rank sum test, and univariate Logistic regression analysis were applied to analyze data. Indicators that were statistically significant in univariate Logistic regression analysis would be analyzed again in non-condition

Table 2

Measure identified in pre-survey for both cases and control groups.

Module	Items
General socioeconomic status	Age, gender, education level, medical insurance status, types of occupation
Daily exposures	Whether living or working near chemical factory or ionizing radiation, whether living in newly decorated apartments/house, having pets/livestock
Individual behaviors	Smoking* (daily number of cigarettes and how many years of smoke), drinking†(daily intake, how many years of being drinking, types of drinking), diet (well balanced, vegetarian, mainly meats, salty‡, preference of sugar§, preference of oil¶, preference of fried food, preference of preserved food), water intake, habit of stay-up late
Occupational exposures	Exposure to harmful substance‖ (including industrial glue, paint, industrial dyes, thinners, grease, uranium mineral, luminescent paint, methanol, formaldehyde, hair dye, heavy metal, dust/coal dust/cotton dust, coal or petroleum, benzene pesticides, chemical fertilizers, organic phosphorus pesticides, organic chlorine pesticides, medical radiation, industrial irradiation, civil aviation radiation, mineral irradiation, nuclear fuel exposure, laser, ultraviolet)
Individual health status	First onset of AA, infection 3 months before AA onset, history of hepatitis, born status (such as, full-term delivery, premature delivery, low birth weight), whether mother exposure to toxic and harmful substances, for female adults: age of first pregnancy, termination of pregnancy, fetal birth status, times of pregnancy till the first onset of AA
Family disease history	Having the same disease, other blood diseases, malignant tumor

AA = aplastic anemia.

* Daily smoking of >5 cigarettes inclusive in the past 3 months was defined as smoking addition in the questionnaire.

† We use the following method to measure daily intake of alcohol and define addiction of alcohol.

Measure of daily alcohol intake (g) = measure of daily intake of liquid with alcohol (mL) > percentage of alcohol in the liquid (%) > 0.8 (ratio of alcohol).

Measure of accumulative alcohol intake (g) = daily alcohol intake (g) > 365 > years of drinking.

Alcohol addition: accumulative alcohol intake over 100 kg, or daily alcohol intake over 60 g for 5 years or above.

‡ Preference of salty food: daily intake of salt over 6 g.

§ Preference of sugar: daily intake of sugar over 40 g.

¶ Preference of oily food: daily intake of oil over 25 g.

‖ Definition of exposure to toxic or harmful substance: daily exposure to chemical toxic (dust/coal dust/cotton dust, coal or oil, industrial glue, industrial dyes, formaldehyde, paint, heavy metals, methanol, etc.) at workplace for 6 to 8 hours, over 3 to 5 years accumulatively; annual exposure to chemical toxic (pesticides containing chlorine, organic phosphorus pesticides, organic chlorine pesticides, fertilizers, and hair dye, etc.) for more than twice, over 3 to 5 years accumulatively. Besides, the frequency of exposure falls into 3 categories: "never" means never or occasionally exposed to the chemic toxic mentioned above; "sometimes" means occasionally exposed but not the exposure has not met the definition of exposure mentioned above; "frequently" means the exposure has met the definition of exposure to chemical toxic in the questionnaire.

multivariate Logistic Regression. ROC was applied to analyze joint contributions of multivariate factors.

3. Results

There were 1802 eligible participants, of which 338 cases and 1464 controls. In the case group, there were 168 men (accounting for 49.70%) and 170 women (50.30%), with median age of 29 (range from 1 to 79) and average age of 32.250 ± 19.022. While in the control group, there were 774 men (52.86%) and 690 women (47.14%), with median age of 28 (range from 1 to 79) and average age of 31.580 ± 19.170. There was no significant difference in sex distribution in both groups. Besides, the education level also had no significant difference. However, in term of medical insurance coverage, the difference in both groups was statistically significant. Obviously, the percentage of patients who were uninsured was 3.03 times higher in the case groups than in the control group, which was statistically significant ($P < .05$). We assume that the uninsured patients paid less attention to their wellness and health care, resulting in delayed detection and treatment of their health problems. Socio-demographic distribution of both groups was presented in Table 3.

3.1. Univariate logistic regression analysis

3.1.1. Daily exposures. Living in a newly decorated house/apartment was found as a risk associated with AA, with odds ratio (OR) of 10.22 ($P < .001$), but durations did not make significant difference. Besides, the presence of both chemical factories within 3 km of the residence and transformers or high-voltage wires within 200 m of the residence was also identified to be associated with AA, with OR of 8.83 ($P < .001$) and of 106.55 ($P < .001$), respectively. Contacts with pets were also significantly associated with AA (OR = 1.61, $P < .001$) (Fig. 1).

3.1.2. Individual behaviors. Several behaviors were identified as risks to be associated with AA, including smoking, staying up late, alcohol drinking, preference of oily, salty, sweet, fried, and preserved food; while 2, that is, balanced diet and drinking tap water, were found to be a protective factor of AA (Fig. 1). Further analysis on smoking status of participants showed that the case group has longer exposure to smoking than the control group ($t = 2.166$, $P = .03$), but the number of cigarettes smoked weekly was not statistically significant. This indicated the positive associate of AA with duration of exposure to smoking, but not with the short-term smoking intake. Alcohol drinking was also a risk, in addition, the risk of AA increased along with the increase of drinking frequency (OR = 1.45, 95% CI: 1.219–1.733, $P < .001$). Further analysis showed that 2 groups had no significant difference in monthly intake of alcohol drinking ($T = 0.694$, $P = .488$) and years of drinking ($T = 0.053$, $P = .958$). Our further analysis found that main reasons for staying up late included: night shift (31.39%), surfing online (25.55%), reading (19.71%), and others (23.36%).

3.1.3. Occupational exposure. The results showed that dust/coal dust/cotton dust (OR = 2.07), organic chlorine pesticides (OR = 2.15), organic phosphorus pesticides (OR = 2.31), industrial glue (OR = 6.41), paint (OR = 14.21), hair dye (OR = 4.44), grease (OR = 15.49), formaldehyde (OR = 12.14), physical radiation (OR = 15.79) were significantly associated with AA (Fig. 1), but others such as chemical fertilizers, coal or petroleum, industrial fuel, heavy metal, and methanol did not show statistical significance.

Table 3

Socio-demographic features of participants in case and control groups (n = 1802).

	Case group (n = 338)		Control group (n = 1464)			
	Number	Percentage (%)	Number	Percentage (%)	z/χ^{2*}	*P*
Gender					0.979	.322
Male	168	49.70	774	52.86		
Female	170	50.30	690	47.14		
Age					0.772	.440
<15 y	78	23.08	366	25.00		
16–35 y	118	34.91	492	33.61		
36–55 y	146	43.20	390	26.64		
≥56 y	63	18.64	216	14.75		
Education level					1.571	.116
Illiterate	30	8.88	158	10.79		
Primary School	102	30.18	479	32.72		
Middle School	100	29.59	400	27.32		
High School	53	15.68	229	15.64		
College Diploma	19	5.62	89	6.08		
BA	30	8.88	101	6.90		
Master's Degree	4	1.19	8	0.55		
Medical Insurance					52.57	<.001
Insured	295	87.28	1396	95.36		
Uninsured	43	12.72	68	4.64		

BA = Bachelor of Art.

* non-parametric rank sum test was used to determine whether the differences in education level and age were statistically significant. While chi-square test was applied in determine the other two indicators.

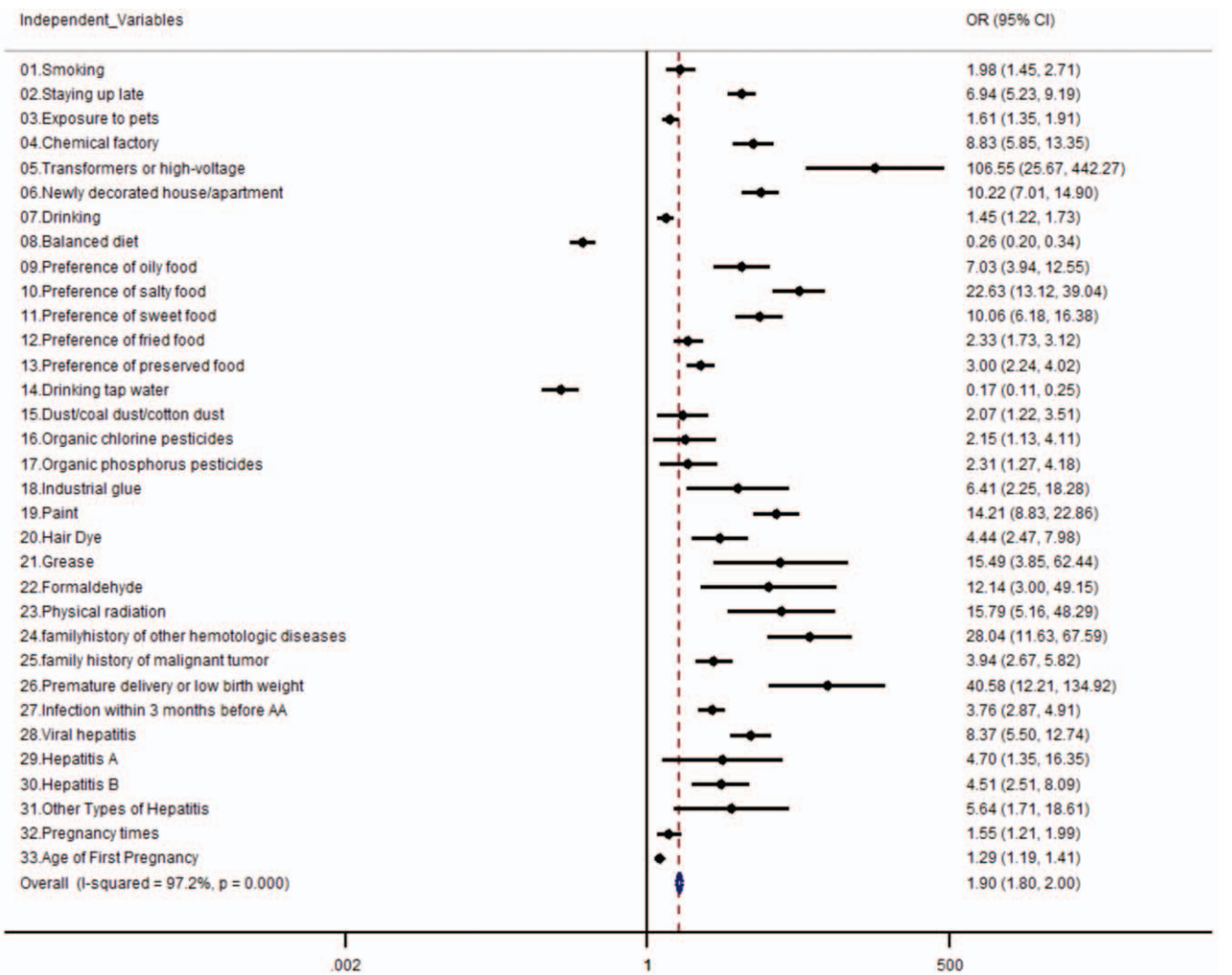

Figure 1. Forest plot showing the effect of each variable against AA in univariate logistic regression analysis. AA = aplastic anemia.

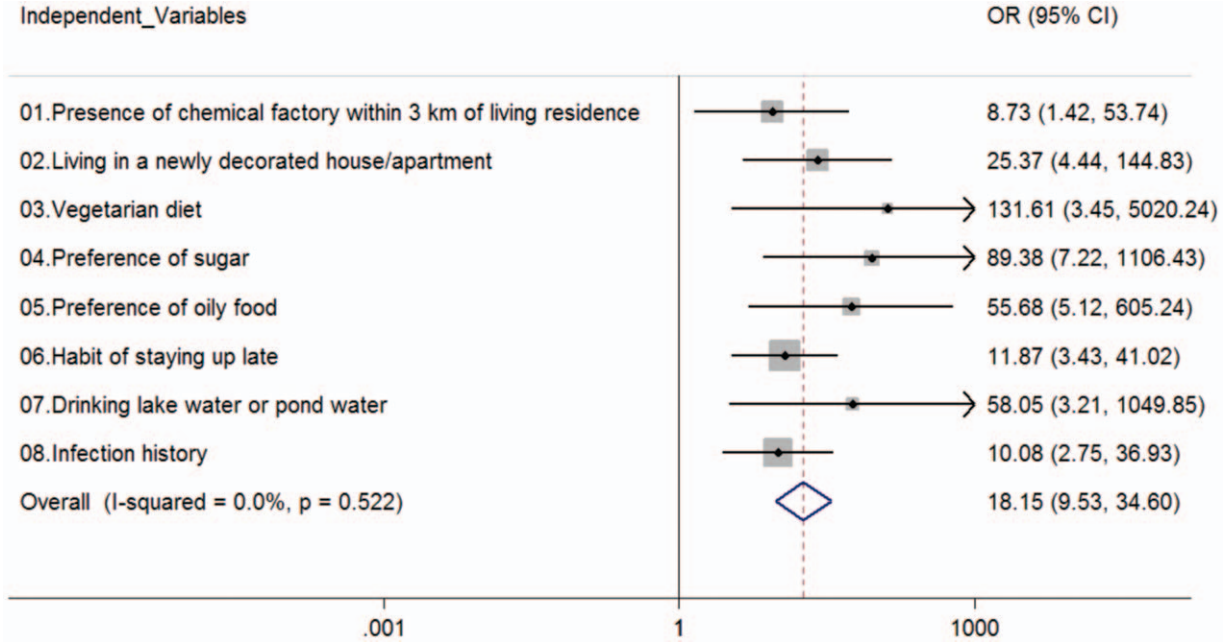

Figure 2. Forest plot showing the effect of each variable against AA in multivariate logistic regression analysis. AA = aplastic anemia.

3.1.4. Individual health status. Viral hepatitis was found to be a risk to AA (OR = 8.37, $P < .001$) (Fig. 1). When analyzed with different types of hepatitis, all types of hepatitis, including hepatitis A, B, and others, showed to be associated with AA, with OR of 4.70, 4.51, and 5.65, respectively. In addition to hepatitis, other infection within 3 months before AA onset was also associated with AA (OR = 3.76, $P < .001$). Premature delivery or low birth weight was also another risk of AA with OR of 40.58.

A separate analysis was conducted among female participants aged between 15 and 49 years old to identify the association between pregnancy and AA. There was no statistical difference in age distribution between case group and control group ($P > .05$), with the average age of 34. The difference of pregnancy times and age of first pregnancy between case group and control group was obviously significant ($P < .001$). The risk of AA increased with the increase of the number of pregnancies and age of 1st pregnancy.

3.1.5. Family disease history. AA was found to be associated with the patients whose parents or grandparents had other hematologic disease (OR = 28.04, $P < .001$). Another risk was malignant tumor in the patient's family members, and the risk of AA in these patients was 3.94 of that in the non-exposed patients (Fig. 1).

3.2. Multivariate logistic regression analysis

The variants which were statistically significant in the above univariate analysis were again included in non-condition multivariate Logistic regression analysis. The analysis results indicated the independent risks causing AA were: presence of chemical factory within 3 km of living residence (OR = 8.73, 95% CI: 1.42–53.74, $P = .019$), living in a newly decorated house/apartment (OR = 25.37, 95% CI: 4.44–144.81, $P < .001$), vegetarian diet (OR = 131.60, 95% CI: 3.45–5020.24, $P = .009$), preference of sugar (OR = 89.38, 95% CI: 7.22–1106.43, $P < .001$), preference of oily food (OR = 55.68, 95% CI: 5.12–605.24, $P = .001$), drinking lake water or pond water (OR = 58.05, 95% CI: 3.21–1049.85, $P < .001$), habit of staying up late (OR = 11.87, 95% CI: 3.43–41.02, $P < .001$), infection history (OR = 10.08, 95% CI: 2.75–36.93, $P < .001$). Please refer to Fig. 2 for details. Several other variants that showed statistical significance in univariate analysis did not show similar result in multivariate analysis, which may be explained by lack of large sample. Their association with AA needed to be further confirmed (Fig. 3).

3.3. Joint contribution analysis

The number of risks that were statistically significant in multivariate Logistic Regression analysis was taken as an independent variable to conduct a Logistic Regression analysis. The results showed that the median number of risks was 2 (ranging 0–5) in the case group, and the median was 0 (ranging 0–6) in the control group. Every additional risk could increase the

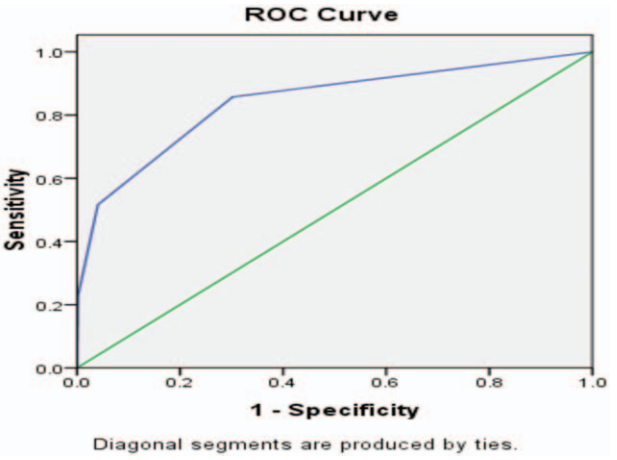

Figure 3. ROC analysis. ROC = receiver operating characteristic.

risk of AA by 6.156 times (95% CI: 5.064–7.484). ROC analysis identified the best cutoff value of 8 risks was 1, when sensitivity plus specificity reached the largest of 1.556 (sensitivity = 0.859 and specificity = 0.302), and area under ROC curve (AUC) reached 0.842 (0.816–0.868, $P < .05$). When categorizing the number of risks into 2 categories of ≥1 and <1, the Logistic Regression analysis results indicated that AA was 13.835 times likely to occur when exposed to ≥1 risks than those exposed to 0 risks (95% CI: 9.995–19.149).

4. Discussion

The identified risks associated with AA could be classified as unpreventable ones, such as individual health status, and preventable ones, such as individual behaviors, daily and occupational exposures, etc. These preventable risks were critical to reduce the incidence of AA and improve people's life quality.

Individual health status concerns individual disease history, family history, and individual's wellbeing, and for women, the pregnancy and delivery status also plays a role. There were evidence to show that >10 viruses could result in AA, including hepatitis virus, Epstein-Barr virus, and B19 virus etc.[12] Some Chinese researchers also reported the high association between influenza virus and AA.[13] Chen explained that virus could activate proliferation of T cell as an antigen, and identify hematopoietic cells with antigen expression to cause autoimmune response, which eventually results in the failure of bone marrow. He also stated that virus could directly depress hematopoietic cells and destroy the micro-circulation of bone marrow, which resulted in chromosome aberration.[14] However, some Chinese studies have showed little association between hepatitis A and AA,[15] and a study in Thailand showed no association identified between hepatitis B and C and AA, except for hepatitis A.[16] The association between hepatitis virus and AA has always been disputable and further studies are needed to address this issue.

Several studies have reported that some genes were closely related with AA, its progression and prognosis, but the exact association between them is unknown. As Wang et al[17] has pointed out in his study that cytochrome P450 2E1 (CYP2E1) (c1c2, c2c2) and glutathione S-transferase mu 1 (GSTM1) might be a genetic susceptible marker, and the patients carrying this gene were more likely to develop AA. Besides, abnormal up-regulation expression of GATA-3 was able to impact on microenvironment of bone marrow and hematopoietic function of AA, which indicated that GATA-3 may be involved in the progress of AA by regulating stromal cell and immunocyte in the microenvironment.[18] These evidences have indicated that to some extent individual's or family genes may play a role in causing AA.

For women, the individual's health status was not only affected by various infection and their genes, but also by their pregnancy status. Pregnancy-related AA, as a special case of perinatal AA, has 2 situations, one is first onset of AA during pregnancy and the other is having AA before pregnancy. According to Cao,[19] pregnancy-related AA accounted for 0.03% to 0.08% of the total pregnancy in China. Young believed that AA was partially associated with pregnancy.[20] And other study reported that a few women had the onset of AA during pregnancy, which turned into remission after delivery but relapsed during the second pregnancy.[21] However, at present, the exact etiology of perinatal AA is still unknown. Influences of hormone changes, malfunction of immune system, or changes in hematopoietic microenvironment may be able to explain.

The unpreventable risks discussed above require more studies to review their association with AA systematically or confirm its etiologic mechanism. Therefore, our attention should be given to the preventable ones, that is, individual behaviors, daily and occupational exposures to toxic substance.

Only a few studies on the association between individual behaviors and AA have been reported before. Our study results indicated that smoking, alcohol drinking, and eating habits were all associated with AA. The identified positive association between AA and duration of exposure to smoking has not been reported yet in published literatures. The association between alcohol drinking and AA may be explained by a study in UK. The study found that as high as 1/3 of Asian population, such as Chinese, lacked aldehyde dehydrogenase that could eliminate acetaldehyde resolved by alcohol; as a result, the hematopoietic stem cells of these people were sensitive to acetaldehyde which could cause irreversible DNA damage in stem cells.[22] This provided evidence that alcohol can damage not only circulating cells but also hematopoietic stem cells. Contact with pets was also associated with AA, but the type of pets needed to be further determined. Our study identified that balanced diet and drinking tap water could protect people being affected by AA, while preference of vegetarian, or meat, or salt, or sugar, or oily food were risks associated with AA. This indicated well balanced diet and drinking clean water was essential to maintain normal function of hematopoietic stem cells and microenvironment, which could be proposed as cost-effective interventions to prevent AA in high-risk population.

In terms of daily and occupational exposures, the identified association with newly decorated house/apartment could be explained by heavy concentration of varied volatile organic compounds (VOCs) and toxic substance such as formaldehyde, benzene, methylbenzene, and dimethylbenzene, etc., in the oil paint that had been released into air during decoration. Our study results confirmed with the previous evidence that long-term exposure to benzene would cause the presence of micronucleus in hematopoietic cell, sister chromatid exchange (SCE), chromosome aberration in nucleus, ever worse to cause AA or other malignant hematologic diseases.[23] In terms of exposure to toxic substance, hair dye was found to be an important risk. There was evidence showed that frequent or long-term use of oxidative hair dyes was one of the hazards related to the hematological tumor.[17] Hair dyes are special cosmetics that contain aromatic amine and nitrogen compound. The genotoxicity study of hair dyes on animal trial result have showed that it has obvious mutagenicity.[24] In the heating process of hair dying, due to its intimate contact with skin, the organic substance of aromatic-amines can easily access capillaries and then to bone marrow through blood circulation. The possible explanation of its association with AA was that, long-term repeated effects of hair dyes on hematopoietic stem cell and microenvironment of marrow may cause damage to the stem cell and change the microenvironment, which led to the occurrence of AA. But its exact mechanism is yet to be known. Other dangerous hazards causing AA occurrence included: pesticides, organic phosphorus pesticides, dust/coal dust/cotton dust, industrial glue, grease, etc.

Although the associations between AA and independent risks have been reported in many studies, pathogenesis of AA is complicated. We assume that AA could be caused by the joint contribution of different independent risks, as AA shows the symptom of deficit of bone marrow stem cells, damage of micro-environment, and malfunction of immunologic system. Our multivariate logistic regression analysis confirmed our hypothe-

sis, and identified the following 8 risks that could jointly cause AA: presence of chemical factory within 3 km of living residence, living in newly decorated house/apartment, vegetarian diet, preference of sugar, preference of oily food, staying up late, drinking lake water or pond water, and infection history. In addition, the analysis on the number of risks showed that every additional risk could increase the risk of AA by 6.156 times. And our ROC analysis also found that AA was 13.835 times likely to occur when exposed to ≥1 risks than those exposed to 0 risks (95% CI: 9.995–19.149). In other words, even if just one exposure of risk was reduced, the likelihood of AA could be reduced greatly. Therefore, it is urgent and important for our government to work out a series of comprehensive interventions against the preventable risks and prevent AA affecting more people.

Patients may have recall bias when reporting or family reports of previous exposures, and that patients in the case group may over report their exposure to explain their disease. In this research, we excluded patients who have died. If we include the dead patients, the information of these patients comes from their family members, which leads to more recall bias. Neiman bias may occur if only including the prevalent case. Therefore, cases diagnosed within recent years that meet the criteria are included in the study reducing the risk of this bias to some degree. And in this study, we tried to reduce the patient-based data bias by selecting objective indicators, selecting appropriate participants, conducting technical training carefully, strictly following the investigator's manual, paying attention to questionnaire questions and survey methods, and data review.

In order to understand the related factors of the incidence of aplastic anemia in Zhejiang Province, as one of the cooperative provinces of "Research on the epidemiological characteristics of aplastic anemia," and due to the shortage of financial input, we selected the hospitalization data of 5 health care centers in 4 representative cities for nearly 10 years. This study used hospital patients as the source of case and control, rather than a random sample of the entire target population, and thus easily led to hospital admission bias. We use a representative multicenter selection study to reduce the bias in the design phase. In future studies, we will continue to increase the research center to further reduce data bias, and study the correlation between each aplastic anemia subtype and risk factors while expanding the sample.

5. Conclusion

Our study results demonstrated that the risks for AA have shifted from the previous single exposure pattern to a comprehensive epidemiological pattern, in which the joint contributions of individual inherited health status, environment exposure, and individual behaviors lead to the occurrence of AA. As mentioned before that AA has trended to increase with increasing environmental pollution and rapidly changing lifestyle in recent year, this pattern is considered to be important and critical to AA's prevention. Based on our study results, the following interventions: early detection of AA high-risk population at community level; early interventions against daily and occupational exposures to toxic substances; early interventions to risky behavior at community level, such as giving up smoking and alcohol drinking, adjusting life schedule, balancing diet, and drinking tap water, etc., are proposed to be taken into practice to prevent the scaling up of AA. Besides, a chronic diseases management network shall be established at community level to provide more effective and high-quality management of chronic diseases. And data collected through this network shall be utilized to monitor the clinical indicators and predict changes of disease progress. This network could provide targeted guidance to AA prevention and treatment and evidence for preventive approaches to avoid AA, which could reduce the disease burden consequently. We expect our study could provide some evidence for preventing and setting up the network of AA management.

Author contributions

Conceptualization: Li-qiang Wu, Sheng-yun Lin.
Funding acquisition: Sheng-yun Lin.
Investigation: Li-qiang Wu, Ying-ying Shen, Yue-min Kuang, Bing-mu Fang, Zhou-zheng Wang, Li-juan Fu, Jian-ping Shen, Yu-hong Zhou, Yi-ping Shen, Bao-dong Ye, Zhi-yin Zheng, Jun-fa Chen.
Methodology: Li-qiang Wu, Ying Zhang, Ke-ding Shao.
Software: Ying Zhang, Ke-ding Shao.
Visualization: Ying Zhang, Ke-ding Shao.
Writing – original draft: Li-qiang Wu.
Writing – review & editing: Sheng-yun Lin.

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