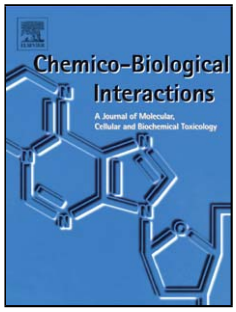
Accepted Manuscript



Title: A Hospital-based Case Control Study of Aplastic Anemia in Shanghai, China

Authors: Sherilyn A. Gross, Richard D. Irons, A. Robert Schnatter, John Ryder, Xiao QinWang, G. Bruce Copley, Thomas W. Armstrong

PII: S0009-2797(09)00560-2
DOI: doi:10.1016/j.cbi.2009.12.015
Reference: CBI 6088

To appear in: *Chemico-Biological Interactions*

Received date: 10-9-2009
Revised date: 10-12-2009
Accepted date: 14-12-2009

Please cite this article as: S.A. Gross, R.D. Irons, A.R. Schnatter, J. Ryder, X. QinWang, G.B. Copley, T.W. Armstrong, A Hospital-based Case Control Study of Aplastic Anemia in Shanghai, China, *Chemico-Biological Interactions* (2008), doi:10.1016/j.cbi.2009.12.015

A Hospital-based Case Control Study of Aplastic Anemia in Shanghai, China

Sherilyn A. Gross [a,c], Richard D. Irons [a,b,c,d], A. Robert Schnatter [e], John Ryder[d], Xiao QinWang [f], G. Bruce Copley[e], Thomas W. Armstrong[g]

[a]Fudan-Cinpathogen Clinical and Molecular Research Center, Institutes of Biomedical Sciences, Fudan University, Shanghai, China; [b]Cinpathogen, Inc., Boulder, CO and Shanghai, China. [c]Molecular Toxicology and Environmental Health Sciences Program, School of Pharmacy, University of Colorado Denver, CO; [d]Department of Pathology, School of Medicine; University of Colorado Denver, CO; [e]ExxonMobil Biomedical Sciences, Inc., 1545 Route 22 East, Annandale, NJ, 08801-0971 USA [f]Huashan Hospital, Fudan University, Shanghai, China. [g]TWA8HR Occupational Hygiene Consulting, LLC

Running Title: Aplastic anemia

Type of Manuscript: Original

Corresponding Author: Richard Irons, Professor & Director
Fudan-Cinpathogen Clin. and Mol. Research Center
(303)724-3558 phone
(303)724-3673 fax
richard.irons@cinpathogen.com

Abstract

We report results of a hospital-based case control study of 137 consecutive patients diagnosed with aplastic anemia (AA) in participating hospitals over a 4 year period. Diagnoses were made by a single laboratory, subjects were age- and gender- matched to two controls and interviewed concerning previous disease, work histories and exposures to potential etiologic agents. Analysis was conducted on two distinct subgroups: severe aplastic anemia (SAA) and moderate aplastic anemia (MAA). In univariate regression models, the strongest associations were observed for exposure to benzene and SAA (OR = 3.12, 95% CI = 1.12-8.65) and life on a farm and MAA (OR = 3.08, 95% CI = 1.44-6.56). Benzene exposure did not show a strong dose-response relationship with either subtype. When accounting for all of the potential confounders we considered in conditional regression models, the previous relationships persisted. Other explanatory variables included hair-dye use for MAA and farm exposures, such as livestock for SAA, although most of these additional variables fell just short of statistical significance. Adjusted r-squared values were only 10% for each subtype, leaving 90% of AA occurrence unexplained. Our results suggest that: (a) benzene exposure is more strongly related to SAA than MAA, (b) farm and livestock exposures are related to both forms of AA, confirming some previous results, and (c) a large percentage of AA remains unexplained, which may indicate that individual susceptibility has a major influence on AA occurrence.

1. Introduction

Acquired aplastic anemia (AA) is a rare disease of bone marrow failure that has been a recognized clinical entity for well over a century [1;2]. The essential features of bone marrow failure in AA are pancytopenia accompanied by bone marrow hypocellularity in the absence of evidence for congenital, neoplastic, or malabsorption disorders, or any other systemic disease or vitamin deficiency. The clinical presentation of AA varies between an acute life threatening illness (i.e. severe) and a progressive debilitating disease (i.e. moderate). Various agents, including numerous drugs, viruses, ionizing radiation and chemicals, most notably benzene, have been implicated in the development of AA. Despite a wealth of literature devoted to this disorder, there are relatively few quantitative epidemiology studies on the etiology of AA until very recently [3]. Historically, a combination of several factors have made the study of AA difficult, including: imprecision in the differential diagnosis of AA as well as misunderstandings and uncertainties concerning the pathogenesis of the disease [4;5]. These issues are further compounded by the fact that AA is by no means the most frequent cause of pancytopenia [6]. The incidence of AA in the general population is extremely low (typically < 1/100,000 worldwide) [7] and even for individuals treated or exposed to known etiologic agents, development of the disease is relatively rare.

Aplastic anemia is commonly thought to be more prevalent in Asia than the West [7]; however, Chinese studies suggest only a modest increase in incidence and no age gender differences relative to the West [8;9]. Reasons for these differences remain unknown but recent studies raise the possibility of an infectious etiology [3;7]. In order to evaluate the prevalence and relative risk of developing AA in Shanghai, China, we conducted a hospital-based case control study of 137 consecutive patients diagnosed with AA from referring hospitals over a 4-

year period. Diagnoses were made by a single laboratory using standardized laboratory and histopathologic criteria. Cases of AA were further classified as chronic (CAA), moderate (MAA) or severe (SAA) to investigate potential etiologic differences for these subtypes. Patients were age- and gender- matched to two controls and interviewed concerning previous history of disease, work histories and exposures to potential etiologic agents.

2. Material and Methods

2.1 Patients

A total of 137 patients were diagnosed with aplastic anemia in our laboratory in Shanghai, China between July 2003 and July 2007. Patients were at least 18 years old and were referred from 28 hospitals in Shanghai. Two controls, for which hematopoietic or lymphoid disease was not suspected as an initial diagnosis, were selected for each AA subject from patients presenting at the referring hospital. Diagnostic categories for control subjects included but were not limited to: trauma (fracture, laceration); infection (pneumonia, rhinitis, bronchitis); cardiovascular disease (hypertension, tachycardia); cancer (gastric, breast, prostate). Controls were successfully matched to 134 patients according to age, gender and date of presentation. Three patients were not assigned hospital controls: one AA patient expired before controls could be selected and two AA patients were lost to contact after diagnosis. Thus, the overall participation rate was 97.8%. Informed consent was obtained on all participating subjects according to the Declaration of Helsinki, 2004 and the NIH Common Rule (45CFR46). This study was approved by the Colorado Multiple Institutional Review Board and the Ethic Committee of Fudan University in Shanghai, China.

2.2 Questionnaire description

The questionnaire and data collection procedures are described in Armstrong et al. 2009 [10]. All cases and controls were interviewed by trained personnel in the hospital setting. In a few cases, subjects were interviewed at home if they had left the hospital prior to interview. The questionnaires used in this study were designed in English, translated into Chinese and then administered in the native Chinese language. Information obtained in the questionnaire included patient demographics, family history of disease, patient medical history (diseases, medications), patient occupational history, exposures of interest associated with each reported job, and patient non-occupational exposure history (e.g. hobbies, smoking, alcohol use). In addition, occupations and industries were coded to Chinese classification systems.

The study questionnaires included segments to evaluate occupational exposures to broad categories of agents and more specific hazards in each of the broad categories. The categories and agents were selected based on reported possible association with lymphohematopoietic diseases (e.g. leukemias and lymphomas) for a suite of studies, including but not specifically the currently reported AA study. The categories were structured so that a relatively non-specific response such as "solvents" could be captured and further detailed (e.g. "chlorinated solvents") if the interviewee knew more specific composition information. The broad categories included: (a) petroleum, products and solvents (18 subcategories), (b) metals (21 subcategories), (c) agricultural chemicals (12 subcategories) and (d) other substances (21 subcategories including ionizing radiation, fumigants, hair dyes). The questionnaire process also solicited responses on the frequency and duration of key tasks involving use of the agents.

Clerical staff entered the data in duplicate from the questionnaires into a database that was verified via an external quality assurance audit. Any disagreements between questionnaire entries were resolved quickly by referring to the original paper record or the questionnaire

administrator. A summary file was generated from the questionnaire data, which did not include any of the clinical information, such as clinical diagnoses, that could compromise blinding. This summary file was assessed and edited via a comprehensive series of logic and consistency checks to assure data integrity.

We combined information from different fields on the questionnaire to define an enhanced and more inclusive variable for analysis. These instances included: (a) information on diabetes and diabetes treatments (i.e. tolbutamide) for a diabetes indicator, (b) information on tuberculosis (TB) and TB treatments for a tuberculosis indicator (c) growing crops indicator which used information on fertilizers, insecticides, pesticides, and growing crops, and (d) an inflammation indicator that combined information on infections and inflammatory diseases such as arthritis, hepatitis and TB.

2.3 Sample collection and clinical laboratory analysis

Peripheral blood, bone marrow aspirates, tissue and core biopsies were collected in conjunction with diagnostic procedures. Peripheral blood smears were obtained by finger stick. Bone marrow aspirates and core biopsies were obtained by Jamshidi needle extraction from the posterior iliac crest. Blood samples were collected by venipuncture and processed for routine CBC (Cell Dyne 3700, Abbott, Abbott Park, IL) and viral serology (HCV and HIV) (Imx, Abbott). Serum vitamin B12 and folate were measured by chemical luminescence (Beckman Coulter Dxi800), and total iron binding capacity was determined using a Beckman Coulter LX20.

2.4 Morphology

Bone marrow aspirate and peripheral blood smears were both evaluated in these cases using Wright-Giemsa stained preparations. Core biopsy sections were evaluated using Hematoxylin-Eosin (H&E), Gomori trichrome, iron and immunoperoxidase-

immunohistochemistry stains for selected markers. Morphology was independently evaluated by two of us (R.D.I., J.R.). Microscopic analysis was performed using Olympus BX51 bright field microscopes (Olympus Optical Ltd, Tokyo). In this study, cellularity was determined by evaluating trephine biopsy sections. However, dysplasia and hypocellularity were also evaluated in bone marrow aspirate smears.

2.5 Cytogenetic and FISH analysis

Cytogenetic studies were performed on either bone marrow or peripheral blood collected at diagnosis. Metaphases were prepared from unstimulated, short-term culture preparations (24- and 48-hour) and G-banded with trypsin-Giemsa staining. A minimum of 20 metaphases were analyzed in each case. Fluorescence *in situ* hybridization (FISH) analysis was performed on short-term cultures of bone marrow or blood cells. Systematic screening for -5/5q-, -7/7q-, +8, del(20q) and 11q23/*MLL* rearrangements was performed on each patient.

2.6 Diagnostic criteria for acquired AA and AA subtypes

The defining morphologic characteristic for diagnosis of AA is a hypocellular bone marrow, typically less than 25%, accompanied by minimal dysplastic changes (see below) and a persistent decrease in peripheral blood cells in at least 2 of 3 lineages [11]. Cases of AA were further classified as severe or moderate based on peripheral blood counts. A diagnosis of SAA was made when 2 or more of the following were present: absolute neutrophil count (ANC) < 500 cells/uL; absolute platelet count (PLT) < 20 X 10^3cells/uL; absolute reticulocyte count < 40 X 10^3cells/uL. A diagnosis of MAA was made when ANC, PLT or absolute reticulocyte counts were persistently decreased below laboratory standard reference ranges but did not fulfill the requirements for diagnosis of SAA. All cases of MAA had involvement of at least 2 lineages. A small number of cases with depressed bone marrow cellularity were accompanied by persistent

but mild depression in only 1 lineage. These were classified as chronic AA. Since there were only five cases of CAA, they were not included in statistical analyses. A diagnosis of acquired AA was not made in patients with accompanying decreases in serum vitamin B12, folate, increased total iron binding capacity or, in cases of pancytopenia, accompanied by normal or increased bone marrow cellularity (≥ 40%). Patients meeting criteria for diagnosis of myelodysplastic syndrome (MDS) were excluded. It is often difficult to distinguish between hypocellular MDS and SAA, and for at least a subset of cases, conceptual differences remain blurred. Therefore, we employed standardized morphologic criteria for the differentiation of SAA and hypocellular MDS. Minor dysplastic changes in erythroid cells were considered consistent with a diagnosis of AA. Alternatively, cases with hypocellular bone marrow accompanied by any evidence of granulocytic or megakaryocytic dysplasia or those with severe dyserythropoiesis, including megaloblastic changes or markedly abnormal nuclear morphology (e.g. nuclear bridging) were not diagnosed with AA.

2.7 Exposure assessment from questionnaire data

The questionnaire that solicited exposure information for cases and controls consisted of a detailed assessment of each patient's work history. This work history queried jobs held over each patient's entire career. Specific jobs elicited more detailed queries to enable the assessment of exposure to classes of hazards [e.g. petroleum products, metals, agricultural chemicals, other (including ionizing radiation)], and when possible, specific substances within each class. It was recognized that many patients would not have detailed knowledge on specific compounds handled, so rather than query specific compounds, the job (and key tasks) scenario was queried in as much detail as possible. This enabled the creation of an exposure summary file from the questionnaire data regarding potential chemical exposure. Subsequent exposure assessment

steps involved at least one, but often several, of the following steps: (a) location of exposure measurement records for the job and location where the subject worked through the Shanghai Institute of Public Health Supervision (IPHS) database, (b) location of records for surrogate factories, industries or jobs in the IPHS database, (c) assessment of exposures detailed in the Chinese literature (supplemented by Western literature) for relevant industries (d) assessment of regulatory and technology changes for key industries, (e) monitoring data from similar facilities/jobs, and/or (f) conducting task simulations. An expert panel integrated the information available and assessed exposures using the above data. The expert panel was comprised of Chinese professors in Occupational Health from Fudan University and a retired director for the Shanghai IPHS. The panel has expertise in exposure to benzene and benzene-containing materials as well as for exposures to others agents covered in the questionnaires. Additional expertise providing discussion and advice to the Chinese experts included an American certified industrial hygienist (CIH) with extensive expertise in exposure assessment for epidemiology. A US trained, Chinese born and raised CIH provided translation, supervision, and technical support as well.

Since benzene exposure was a focus of the study, a semi-quantitative approach toward exposure assessment was employed. Five ordinal categories of exposure were created: (0) no occupational exposure, (1) < 1 mg/m^3, (2) 1 - <10 mg/m^3, (3) 10 - <100 mg/m^3, and (4) 100+ mg/m^3. Each job/location scenario was placed into one of these five categories using the procedures summarized above. We assigned category 1 to a 'score' of 1, category 2 a 'score' of 2, etc. For some analyses, the score assigned to a specific exposure category was multiplied by the number of months in that category to calculate an index of 'score-months' of benzene exposure. This index was categorized into tertile categories according to the distribution in

controls, and these categories, as well as the continuous measure of score-months, were used in conditional logistic regression models. The benzene exposures included contributions from selected materials such as some solvents, fuels, glues, paints, etc. that typically contained benzene. Note that not all formulations of materials in these categories were deemed to contain benzene (e.g. fish based glues, latex paints).

For exposures other than benzene, we assigned study subjects a binary indicator to reflect the presence or absence of the substance for a specific job/workplace scenario. No attempt was made to estimate an approximate concentration associated with that scenario. The sources used to determine assignment to "ever" and "never" exposed categories included the aforementioned Chinese literature searches for relevant industries, Western literature [12;13] and knowledge of a Shanghai-based expert panel. Finally, to explore whether a diversity of chemical exposure might have a role in AA, we summed the number of positive indicators for each chemical exposure and used the sum as a covariate in exploratory regression models, described below.

2.8 Statistical analysis

The primary analytic tool used was conditional logistic regression analysis. Separate models were fit for both MAA and SAA. We first examined univariate conditional logistic regression models with grouping for the matching variables of age and gender. Since model stability was a concern, especially for conditional logistic regression models, variables were assessed for co-linearity to facilitate subsequent covariate selection for the multivariate conditional logistic regression models. Model stability was further enhanced by removing or collapsing some highly correlated component covariates (e.g. the category 'fuels' was derived from exposure indicators to diesel, gasoline and kerosene because each variable correlated with 'fuels' and with one another). However, statistically distinctive component variables were

preferentially retained, pending further assessment, over their broader "parent" data elements when the latter was uninformative.

As with the univariate analyses, multivariate models were conditioned on the matching factors of age and gender. Parsimonious models were built using a step-wise process that iteratively eliminated covariates if the p-value was > 0.2 and subsequently allowed for covariate re-entry after each successive run if the p-value was < 0.10. In addition to the matching factors, the Odds Ratios (OR) from these models are controlled for simultaneous effects of other factors deemed important predictors in explaining SAA and MAA risk. If conditional models would not converge for a key potential predictor, unconditional models were used. Important predictors were determined by examining the effect of exchanging model terms unrelated to model stability. Residual analysis was assessed by both Akaike's Information Criterion and the Bayesian Information Criterion, which also guided the selection of predictors in final models. Gender-specific models were also explored. All statistical analyses were performed using Stata Statistical software: Release 10 (Stata Corp. College Station, TX).

3. Results

3.1 Clinical characterization of AA and AA subtypes

Of the 134 total AA cases that participated in this hospital-based case control study, the proportion of cases diagnosed as SAA, MAA or CAA are presented in Table 1. In addition to the peripheral blood parameters (Table 2) and bone marrow morphology described for the classification of AA subtypes, cytogenetic and/or FISH analysis were also performed and were successful in 114 out of 134 patients (85%). However, no clonal cytogenetic abnormalities were observed in any of the cases diagnosed with AA using criteria as described in Materials and

Methods. Age and gender distributions for all 134 cases of AA are illustrated in Figure 1. Overall there was a slight male predominance: 63 females and 71 males presenting with some form of AA. The median age for males was 36 years (range 18-87 years) whereas the median age for females was 43 years (range 18-85 years). There were significantly more males than females in the $\leq$ 29 year old age group ($p < .05$), which is partially explained by a marked increase in young males presenting with SAA (Table 1). There were 19 males but only 7 females under the age of 30 years diagnosed with SAA. Males also exhibited a tendency towards diagnosis of SAA versus MAA in the 60-69 year age group (7 cases versus 1 case, respectively). However the increase was not statistically significant. Females presented with a relatively even distribution of SAA and MAA throughout the different age groups except for the seventh decade of life. Seven cases of MAA were diagnosed in women age 70-79 years whereas only 1 case of SAA was diagnosed in this same age group (data not shown).

3.2 Risk factors associated with the development of AA and AA subtypes.

Risk factors as determined in univariate models associated with the development of AA and its subtypes are summarized in Table 3. For SAA, the only significant positive finding was benzene exposure when it was classified as "ever/never" exposed (OR = 3.12). In addition, when the highest benzene score for each study subject was regressed against SAA, the OR was 1.56 (95% CI = 0.99 – 2.47), a result that is not quite statistically significant ($p = 0.056$). The OR of 1.56 indicates a 1.56- fold increase in relative risk for each incremental category of exposure (i.e. < 1 mg/m^3, 0.1 – 1 mg/m^3, 1 – 10mg/m^3, etc.) (data not shown). However, the score-months index showed no relationship with SAA (OR = 1.00). This indicates that multiplication of the benzene score by the duration of benzene exposure did not produce a higher

risk for SAA. Other suggestive findings for SAA included exposure to fuels, exposure to cutting oils and exposure to livestock, although each of these fell just short of statistical significance.

For MAA, the only significant risk factor was for living on a farm (OR = 3.08), which was highly statistically significant ($p < 0.004$). The OR for "ever" exposed to benzene was surprisingly only 1.22 and not significant. Furthermore, benzene score-months was not significantly related to MAA (OR = 0.98), nor was the highest benzene score (OR = 1.15, 95% CI = 0.70 – 1.88, $p = 0.57$) (data not shown).

Significant risks ($p < .05$) were identified for total AA cases associated with a history of exposure to fuels (OR = 2.35, 95 % CI = 1.02-5.42) and/or living on a farm (OR = 2.05, 95 % CI = 1.28-3.28). Both exposure to cutting oils (OR = 2.50, 95 % CI = 0.99-6.33) and glues (OR = 3.50, 95 % CI = 1.02-11.96) were not quite statistically significance ($p = 0.05$). There was a marginal increase ($p = 0.06$) in the risk of developing AA associated with exposure to benzene (OR = 2.0, 95% CI = 0.97-4.13), but that was due to the aforementioned significant risk for SAA (OR = 3.12). There was no significant risk for the development of AA associated with exposure to other solvents, cleaning agents, hair dyes or use of traditional Chinese herbs. Similarly, microbial agents, a medical history of hepatitis, tuberculosis, or inflammatory disease did not explain an increased risk in the development of AA.

3.3 Multivariate analysis of AA subtype

We attempted to fit conditional models for the highest benzene score and benzene score-months variables (Table 4). This was only partially successful, as some models would not converge. There is a suggestion of a monotonic relationship between highest benzene score and MAA, although no categories are statistically significant. The score-months variable would not converge.

For SAA, there is a "U" shaped dose-response for benzene when classified according to the highest score, with the lowest and highest scores showing identical OR's of 6.0 (Table 4). Quite unexpectedly, the score-months index showed a negative relationship with SAA, though this was not statistically significant. For both SAA and MAA, the highest benzene score seems to be a better predictor than score-months, indicating that long exposure durations do not necessarily portend a higher risk for the development of AA.

A conditional logistic regression analysis was used to identify the variables that best explain the development of MAA or SAA subtype. Both models achieved an adjusted R^2 of approximately 10% for either MAA or SAA subtype and a summary of these findings are presented in Tables 5 and 6, respectively. Adjusting for all other variables in our questionnaire, life on a farm was still the best predictor for the development of MAA (OR = 3.08, 95% CI = 1.43-6.62). Hair-dye use also was retained in the final model, although it fell short of statistical significance (p = 0.12) (Table 5).

In contrast, exposure to benzene was the best predictor for the development of SAA. A simple definition of "ever/never" exposed to benzene performed best, indicating either misclassification of exposure for highest score, or a lack of importance between estimated intensity and duration of exposure and SAA risk. There were other notable predictors retained in the final model, including living on a farm, exposure to livestock, body mass index and diabetes. The latter two predictors had a protective effect (Table 6).

Analysis between main exposure covariates revealed a moderate correlation between exposure to benzene and gasoline, paint and the solvents toluene/xylene. There is a strong correlation between exposure to organophosphates and exposure to fertilizer, pesticides and

growing crops. Exposure to microbial agents correlated with the use of cleaning agents as well (data not shown).

In order to determine if there are gender-specific risk factors associated with the development of a specific AA subtype, conditional logistic regression analysis was used to identify risk factors that best explained the development of MAA in males (Table 7), MAA in females (Table 8) and SAA in males (Table 9). Gender stratification was attempted for development of SAA in females but model instability precluded meaningful statistical analysis of that endpoint. For development of MAA in males, the use of hair dye emerged as a significant risk factor (OR = 11.8, p = 0.007, 95%CI = 1.96-71.6) (Pseudo R^2 = 28%) while life on a farm remained the most persistent explanatory variable for the development of this disease subtype in females (OR = 5.4, p = 0.009, 95% CI = 1.5-19.8) (R^2 = 14%). Surprisingly, there was a significant inverse association for multiple exposures to non-benzene chemicals in males (OR = 0.13, p = 0.01, 0.02-0.64). This finding suggests that male cases had less diverse employment resulting in fewer chemical exposures versus controls. This finding is likely to be either the result of multiple hypotheses testing or related to the fact that there were few significant relationships noted between MAA and the occupational agents under study. In contrast, several risk factors emerged as significant explanatory variable for the development of SAA in males (Pseudo R^2 = 28%) (Table 9). Most notable was benzene "ever/never" exposed (OR = 8.3, p = 0.02, 95% CI 1.42-49.3) followed by exposure to pesticides (OR = 6.1, p = 0.03, 95% CI 1.13-33.0) and a history of hepatitis (OR = 6.8, p = 0.05, 95% CI 1.00-46.5). Body mass index and exposure to cutting oils remained in the model albeit were not statistically significant.

Discussion

Over the previous century, most of the information concerning the etiology of acquired aplastic anemia has been anecdotal. A vast number of agents have been variously hypothesized to cause AA including antibiotics, anti-diabetic agents, analgesics and other therapeutic agents, use of medicinal herbs, use of hair dyes, and occupational exposure to pesticides, herbicides, fertilizers or farming, exposure to benzene as well as viruses and inflammation. However, until recently, there was virtually no quantitative evidence linking risk of exposure to any of these factors with the development of AA. In 2006, Issaragrisil and colleagues reported the results of a large epidemiologic study of AA conducted in Thailand [3]. This was also a hospital-based, case-control study designed to estimate incidence rates as well as risks of various environmental exposures on AA occurrence. The results of this study confirmed significant risks of AA associated with exposure to benzene and work in agriculture, with notable associations for various pesticides, including organophosphates, non-bottled drinking water, and livestock, (e.g. ducks and geese). Remarkably, no association was observed between post-hepatitic liver disease and the development of AA in general, which has been reported to account for approximately 10% of cases the West [14;15].

Strengths of our study include standardization and precision of diagnosis by a single laboratory, the specification of AA subtypes, a large number of prospectively recruited cases and closely matched controls which were statistically analyzed as such in a conditional logistic regression model. The risks associated with a relatively large number of potential etiologic agents were determined. The strongest associations were observed for exposure to benzene and SAA and life on a farm and MAA. It is interesting that different risk factors emerged for individual subtypes of AA and appear to be gender specific, which suggests that these clinical subtypes may have distinct etiologies. A relatively large number of subjects (40) reported use of

medicinal herbs, although specific agents were only rarely identified. Medicinal herb use was marginally, though not significantly elevated for development of SAA, but no such result was found for MAA. We initially differentiated between SAA and MAA because of our experience that these two conditions present clinically with different severity, respond differently to therapy and are associated with a different clinical course. Therefore, the distinguishing diagnostic criteria have prognostic value. In further observations, SAA was found to be associated with benzene exposure in general and in males. MAA on the other hand, was found to be associated with farming in females and the use of hair dye in males. Taken together, these unexpected findings suggest that SAA and MAA differ with respect to etiology as well as clinical presentation and prognosis, and therefore should be classified as separate entities. Further studies are necessary to confirm and extend these observations.

While benzene has been linked to AA for decades, there is surprisingly little quantitative data on the relationship with AA. Our exposure assessment for benzene was derived from questionnaire responses, along with a detailed assessment of the presence and degree of benzene exposure in the jobs and industries observed from our work history data. This led to the development of ordinal exposure categories that were further assessed by independent scientists using various ranking schemes. We feel the scores represent an accurate assessment of relative benzene exposure in diverse Chinese industries, and should be considered one of the strongest exposure assessments for benzene in the AA literature to date. While we are confident that the relative degree of benzene exposure is represented in the scoring scheme used, we are more cautious regarding precise exposure concentrations assigned to each score. With this in mind, the measure of cumulative exposure we felt was most justified was that of 'score-months', rather than assigning a midpoint concentration to each category in order to calculate a measure

analogous to 'ppm-months'. An additional strength of our exposure assessment is the consideration of other hazards of possible etiologic interest. However, as with most retrospective exercises, the exposure scores are subject to misclassification, which, if random, would be expected to dilute risk towards the null value. While this possibility cannot be excluded, we think that the data illustrated here is valuable in providing insight on the likely magnitude of risk in benzene exposed workers, especially for different AA subtypes.

Hepatitis, which has been commonly linked with the development of AA in the West, was generally not a significant predictor for AA as the OR was non-significant for both MAA (OR = 1.8) and SAA (OR = 1.2). However, hepatitis did emerge as a potential risk factor in males with SAA, where we observed an OR of 6.8 (p = 0.05). Thus, there is some support in our data that the risk of AA associated with hepatitis is both gender and subtype specific. When we looked at a combined grouping of diseases associated with inflammatory or infectious conditions in our patients (i.e. arthritis, hepatitis and TB) we also failed to demonstrate a significant relationship with the development of AA.

There is equivocal support for previous risks that have variously been reported in the literature, a notable example being hair dyes. Since the first report in 1935 [16], a total of 13 cases with a possible link to hair dye has been reported in the literature [16-19]. Overall, we found the risk of developing AA associated with exposure to hair dyes was not significantly elevated (OR = 1.30, 95% CI = 0.80-2.10) despite the wide use of these products in the population (57 cases and 104 controls). Nonetheless hair dye use was retained as a final predictor in the model for MAA, explaining a minor proportion of variance in AA risk. Also, when risk factors were examined by gender, use of hair dye emerged as a large, statistically significant predictor of the development of MAA in males (OR = 11.8, p = 0.007, 95% CI 1.96-

71.6). This may be explained, in part, by the fact that in China the use of hair dye by males is much more frequent than in the West.

Subjects were specifically queried regarding the use of a large number of medications previously identified as potential causes of AA (e.g. chloramphenicol, tolbutamide, sulfonamides, isoniazid, and others) [20]. However, only 18 subjects reported taking medications, with responses largely restricted to tolbutamide and isoniazid, and no risk of developing AA was identified with the use of these two agents. Only one subject reported the use of chloramphenicol, reflecting the restricted indications for use of this drug in current practice in China. Conditional logistic regression analysis revealed a particularly striking negative association between diabetes and development of SAA with an OR of 0.09 ($p < .07$), which remains puzzling and unexplained.

Agricultural chemical exposure was assessed via the structured questionnaires. The responses allowed discrimination of workers who reported using agricultural chemicals in general or more specific materials (fertilizers, herbicides, insecticides) and those that did not. However, the agricultural workers had very limited knowledge about the specific composition of chemicals they used. This aspect limits the information about the herbicides and pesticides used in the general agricultural chemical category.

Since the late 1800's approximately 1500 cases of benzene poisoning have appeared in the world literature [21-31]. Pancytopenia was described in approximately 250 of these cases, the majority of which were assumed to represent AA. Nevertheless, for the vast majority if not all of these cases, examination of bone marrow biopsy sections was virtually undocumented in living patients. Diagnostic precision in these cases was further muddled by the fact that as early as 1939, bone marrow hypercellularity had been reported in the majority of post-mortem cases

associated with benzene-induced pancytopenia [23;24]. Therefore, up until the recent publication of Issaragrisil et al [3] and now this report, the quantitative risk of developing AA following benzene exposure has remained elusive. In our study, the risk associated with benzene exposure was statistically significant only for SAA ($p < 0.03$), particularly in males. A potential explanation may be forthcoming from the unique stringency we placed on the differential diagnosis of AA versus MDS in this study. Previous studies in our laboratory have identified MDS as a frequent outcome in patients with previous exposure to benzene [32]. Taken together with the historical differences in diagnostic precision reported in the early literature, these findings raise the prospect that many cases of AA previously attributed to benzene would today be classified as MDS.

The criteria for differential diagnosis of AA and MDS not withstanding, there is compelling evidence to indicate an important, if not obligatory, role for immune-mediated mechanisms involved in bone marrow suppression in the pathogenesis of acquired AA as well MDS. Building on initial observations that treatment with anti-thymocyte globulin (ATG) or cyclosporine was of clinical benefit in AA patients [33-36], a robust literature has emerged to implicate immune and inflammatory mechanisms in the evolution of the disease [36-40]. In large measure these are findings shared between AA and hypocellular MDS [41], and support a common or at least overlapping etiology in the development of these two conditions [5].

Acknowledgements

We would like to extend our appreciation to Ann Louden for manuscript and clerical assistance and Anh Le, Qian Chen, Gail Jorgensen, Susan Marcella and Allan Holsomback for database management as well as Yimei Zhou and the exposure assessment expert panel

(especially Youxin Liang, Chi Zhang and Xipeng Jin) for their contributions. We would also like to thank the participating hospitals including Huashan Hospital, Xinhua Hospital, Long March Hospital, Huang Pu Central District Hospital, Renji Hospital, Ruijin Hospital, Huadong Hospital, Jin An Central Hospital, No. 1 People's Hospital, No. 5 People's Hospital, No. 6 People's Hospital, No. 9 People's Hospital, Yang Pu Central Hospital, Zha Bei Central Hospital, Shu Guang Hospital, Chang Ning Central Hospital, Tong Ji Hospital, Shong Jin Central Hospital, Zhong Shan Hospital, Railway Hospital, Rong Hua Hospital, Changhai Hospital, Occupational Disease Hospital, Jiading Central Hospital, 455 Hospital, Shidong Hospital, No. 1 Baoshan Hospital, and Putuo Central Hospital. This work was conducted as part of the Shanghai Health Study and was funded by the Benzene Research Health Consortium consisting of BP Amoco, Chevron Texaco, ExxonMobil, Conoco Philips and Shell Chemical Companies.

References

[1] P. Ehrlich. Ueber einem Fall von mit Bemerkungen über regenerative Anami Veränderungen des Knochenmarks. Veränderungen des Knochenmarks, Charité-Annalen 13 (1888) 301-309.

[2] M. Chauffard. Un cas d'anémie pernicieuse aplastique, Bull Soc Med Hōp Paris 21 (1904) 313-315.

[3] S. Issaragrisil, D.W. Kaufman, T. Anderson, K. Chansung, P.E. Leaverton, S. Shapiro, N.S. Young, The Epidemiology of Aplastic Anemia in Thailand, Blood 107 (2006) 1299-307.

[4] W. Thompson, M Richter, K Edsall. An analysis of so-called aplastic anemia, Am.J.Med.Sci. 187 (1934) 77-88.

[5] J. Barrett, Y Sauntharajah, J Molldrem. Myelodysplastic syndrome and aplastic anemia: Distinct entities or diseases linked by a common pathophysiology?, Semin.Hematol. 37 (2000) 15-29.

[6] M. Keisu, A. Ost, Diagnoses in patients with severe pancytopenia suspected of having aplastic anemia, European Journal of Haematology 45 (1990) 11-4.

[7] N.S. Young, B.P. Alter. Aplastic Anemia Acquired and Inherited in: Aplastic Anemia Acquired and Inherited W.B. Saunders Company, Philadelphia:1994; 24-31.

[8] J.P. Zhang, J.H. Xu, X.C. Zhang et al. The epidemiology survey of aplastic anemia in Nin Xia Hui District, Zhong Hua Xue Ye Xue Za Zhi 20 (1999) 211-212.

[9] G.B. Chen. The epidemiology of aplastic anemia, Zhong Hua Xue Ye Xue Za Zhi 20 (1999) 221-222.

[10] T. Armstrong,Y. Zhou, C. Zhang, S. Bowes, Y. Liang, O. Wong, F. Hua, Exposure assessment for a case-control epidemiology study based in Shanghai, China: Summary of methods and results, Chem.Biol. Interact (2009), doi:10.1016/j.cbi.2009.11.008.

[11] J. Maciejewski, A. Rissitano. Aplastic Anemia: management of adult patients, Hematology (Am Soc Hematol Educa Program) (2005) 110-117.

[12] W.A. Burgess, Recognition of health hazards in industry: A review of materials and processes in: Burgess WA. Recognition of health hazards in industry: A review of materials and processes. John Wiley and Sons,New York:1995.

[13] National Institute for Occupational Safety and Health, Occupational Diseases: A Guide to Their Recognition, (1977) 1-50.

[14] K.E. Brown, J. Tisdale, A.J. Barrett, C.E. Dunbar, N.S. Young, Hepatitis-Associated Aplastic Anemia, New England Journal of Medicine 336 (1997) 1059-64.

[15] R. Safadi, R. Or, Y. Ilan, E. Naparstek, A. Nagler, A. Klein, M. Ketzinel-Gilaad, K. Ergunay, D. Danon, D. Shouval, E. Galun, Lack of Known Hepatitis Virus in Hepatitis-Associated Aplastic Anemia and Outcome After Bone Marrow Transplantation, Bone Marrow Transplantation 27 (2001) 183-90.

[16] C. Baldridge, Macrocytic anemia with aplastic features following the application of synthetic organic hair dye, Am.J.Med.Sci. 189 (1935) 759-765.

[17] J.E. Hopkins, A. Manoharan, Severe Aplastic Anaemia Following the Use of Hair Dye: Report of Two Cases and Review of Literature, Postgraduate Medical Journal 61 (1985) 1003-5.

[18] D.M. Williams, G.E. Cartwright, Drug-induced aplastic anemia, Semin.Hematol. 10(3) (1973) 195-223.

[19] C. Huguley, B. Bergsagal, W. Best, E. Beutler, W. Crosby, A. Erslev , N. Schulman, Council on drugs, (April-May 1965).

[20] H. Heimpel, W. Heit, Drug induced aplastic anemia: clinical aspects, Clin.Haematol. 9(3) (1980) 641-662.

[21] L. Selling, Benzol as a leucotoxin: studies on the degeneration and regeneration of the blood and haematopoietic organs, J.H.Med.Rep. 17 (1916) 83-142.

[22] F.T. Hunter, Chronic exposure to benzene (benzol). II. The clinical effects, J.Ind.Hyg.Toxicol. 21 (1939) 331-354.

[23] L.A. Erf, C.P. Rhoads, The hematological effects of benzene (benzol) poisoning, J.Ind.Hyg.Toxicol. 21 (1939) 421-435.

[24] T.B. Mallory, E.A. Gall, W.J. Brickley, Chronic exposure to benzene (benzol). III. The pathologic results, J.Ind.Hyg.Toxicol. 21(8) (1939) 355-377.

[25] L.J. Goldwater, Disturbances in the blood following exposure to benzol, J.Lab.Clin.Med. 26 (1941) 957-973.

[26] R. Wilson., Benzene poisoning in industry, The journal of laboratory and clinical medicine (1942) 1517-1521.

[27] M. Savilahti, Over 100 cases of benzene poisoning in a shoe factory, Arch. Gewerbepathol Gwerebehyg 15 (1956) 147-157.

[28] M. Aksoy, K. Dincol, S. Erdem, T. Akgun, G. Dincol, Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene., British Journal of Industrial Medicine. 29 (1972) 56-64.

[29] E.C. Vigliani, A. Forni. Benzene and leukemia, Environ.Res. 11 (1976) 122-127.

[30] S. Laskin, B.D. Goldstein, Benzene toxicity: a critical evaluation, J.Toxicol.Environ.Health 2 (1977) 1-148.

[31] M.M. Brandao, M.A. Rego, L. Pugliese, J. Clarencio, C.M. Bastos, J. Ferreira, R. Meyer, M. Neves, S.M. Freire, Phenotype Analysis of Lymphocytes of Workers With Chronic Benzene Poisoning, Immunology Letters 101 (2005) 65-70.

[32] R.D. Irons, L. Lv, S.A. Gross, X. Ye, L. Bao, X.Q. Wang, J. Ryder, T.W. Armstrong, Y. Zhou, L. Miao, A.T. Le, P.J. Kerzic, W. Ni, H. Fu, Chronic exposure to benzene results in a unique form of dysplasia, Leukemia Research 29 (2005) 1371-80.

[33] N.S. Young, Autoimmunity and its treatment in aplastic anemia, Ann.Intern.Med. 126 (1997) 166-168.

[34] N. Frickhofen, SJ Rosenfeld, Immunosuppressive treatment of aplastic anemia with antithymocyte globulin and cyclosporine, Semin.Hematol. 37 (2000) 56-68.

[35] B. Speck, E. Gluckman, H.L. Haak, J.J. Van Rood, Treatment of aplastic anaemia by antilymphocyte globulin with and without allogeneic bone-marrow infusions, Lancet (1977) 1145-1148.

[36] S. Nakao, H. Takamatsu, T. Chuhjo, M. Ueda, S. Shiobara, T. Matsuda, T. Kaneshige, H. Mizoguchi, Identification of a specific HLA class II haplotype strongly associated with susceptibility to cyclosporine-dependent aplastic anemia, Blood 84 (1994) 4257-61.

[37] H. Kook, W. Zeng, C. Guibin, M. Kirby, N.S. Young, J.P. Maciejewski, Increased cytotoxic T cells with effector phenotype in aplastic anemia and myelodysplasia, Experimental Hematology. 29 (2001) 1270-7.

[38] A.M. Risitano, J.P. Maciejewski, S. Green, M. Plasilova, W. Zeng, N.S. Young, In-vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenetic T-cell clones by TCR beta-CDR3 sequencing., Lancet. 364 (2004) 355-64.

[39] W. Zeng, J.P. Maciejewski, G. Chen, N.S. Young, Limited heterogeneity of T cell receptor BV usage in aplastic anemia., Journal of Clinical Investigation. 108 (2001) 765-73.

[40] J. Ascensao, R. Pahwa, W. Kagan, J. Hansen, M. Moore, R. Good, Aplastic Anaemia: Evidence for an Immunological Mechanism, Lancet 1 (1976) 669-71.

[41] W. Zeng, G. Chen, S. Kajigaya, O. Nunez, A. Charrow, E.M. Billings, N.S. Young, Gene expression profiling in CD34 cells to identify differences between aplastic anemia patients and healthy volunteers, Blood. 103 (2004) 325-32.

Table 1. Gender distribution of AA subtypes

Gender Distribution of AA Subtypes			
Subtype	**Total (%)**	**Male**	**Female**
SAA	73 (54 %)	42	31
MAA	56 (42%)	26	30
CAA	5 (4%)	3	2

Table 2. Peripheral blood parameters associated with AA subtypes

Laboratory Parameters for AA Subtypes							
	WBC	**ANC** [1]	**ALC** [1]	**PLT**	**RBC**	**MCV**	**Retic** [1]
SAA	1.4 (0.2-3.5)	0.2 (0.01-1.1)	0.8 (0.05-3.1)	16.7 (0-73.5)	1.8 (0.9-4.2)	94.0 (82.6-118)	25.0 (4.0-112)
MAA	2.3 (0.8-6.3)	1.0 (0.2-4.7)	1.0 (0.1-2.7)	37.0 (3.8-370)	2.5 (1.0-4.8)	102.0 (83.4-123)	56.5 (10.7-126)
CAA	4.3 (2.5-5.3)	2.1 (1.1-3.7)	0.9 (0.9-1.5)	114.0 (97.4-160)	3.5 (1.5-3.9)	102.0 (96.2-104)	109.0 (44-195)

WBC White Blood Cells (4-10 x 10^9/L)
ANC Absolute Neutrophil Count (2-7 x 10^9/L)
ALC Absolute Leukocyte Count (1.6-6 x 10^9/L)
PLT Platelets (100-300 x 10^9/L)
RBC Red Blood Cell (4-5.5 x 10^9/L)
MCV Mean Corpuscular Volume (80-100 fL)
Retic Absolute Reticulocyte Count (25-75 x 10^9/L)

Select laboratory parameters displayed as median and range for AA subtypes [1] There are no lab values available in 3 subjects in the clinical dataset.

Table 3. Risk factors associated with the development of AA and AA subtypes

Table 3: Univariate Analysis of Risk Factors Associated with SAA, MAA and All AA												
	SAA				MAA				All AA			
Risk Factor	**#Num. Cntl/Case**	**Odds Ratio**	**p-value**	**95% CI**	**#Num. Cntl/Case**	**Odds Ratio**	**p-value**	**95% CI**	**#Num. Cntl/Case**	**Odds Ratio**	**p-value**	**95% CI**
Benzene (ever/never)	No 139/63 Yes 7/10	3.12	0.03*	1.12-8.65	No 100/49 Yes 12/7	1.22	0.72	0.42-3.52	No 249/117 Yes 19/17	2.00	0.06	0.97-4.13
Benzene (score-months)	NA	1.00	0.62	0.99-1.00	NA	1.00	0.52	0.99-1.01	NA	1.00	0.50	0.98-1.01
Fuels (gasoline, diesel, kerosene)	No 140/65 Yes 6/8	2.94	0.06	0.95-9.10	No 106/52 Yes 6/4	1.37	0.64	0.36-5.29	No 256/121 Yes 12/13	2.35	0.04*	1.02-5.42
Cutting oils	No 142/67 Yes 4/6	3.00	0.09	0.85-10.63	No 108/52 Yes 4/4	2.00	0.33	0.50-8.00	No 260/124 Yes 8/10	2.50	0.05	0.99-6.33
Pesticides, organophos-phates, herbicides	No 132/63 Yes 14/10	1.51	0.36	0.63-3.64	No 99/48 Yes 13/8	1.34	0.58	0.47-3.83	No 240/115 Yes 28/19	1.48	0.25	0.76-2.88
Growing crops	No 121/57 Yes 25/16	1.53	0.39	0.58-4.04	No 93/42 Yes 19/14	2.06	0.19	0.69-6.15	No 222/103 Yes 46/31	1.64	0.17	0.81-3.34
Living on farm	No 87/37 Yes 59/36	1.58	0.16	0.83-2.99	No 72/23 Yes 40/33	3.08	0.004*	1.44-6.56	No 165/63 Yes 103/71	2.05	0.003*	1.28-3.28
Raising livestock	No 139/65 Yes 7/8	3.78	0.06	0.95-15.04	No 103/52 Yes 9/4	0.87	0.83	0.24-3.11	No 252/122 Yes 16/12	1.73	0.22	0.72-4.20
Fertilizers	No 135/64 Yes 11/9	1.94	0.21	0.69-5.48	No 99/48 Yes 13/8	1.37	0.57	0.46-4.11	No 243/117 Yes 25/17	1.55	0.25	0.74-3.24
Paints	No 143/70 Yes 3/3	2.00	0.40	0.40-9.91	No 106/50 Yes 6/6	2.38	0.19	0.65-8.74	No 259/125 Yes 9/9	2.22	0.12	0.82-6.08
Welding, soldering, cutting	No 145/71 Yes 1/2	4.00	0.26	0.36-44.11	No 110/53 Yes 2/3	3.00	0.23	0.50-17.95	No 265/129 Yes 3/5	3.33	0.10	0.80-13.95
Metal machining	No 138/71 Yes 8/2	0.50	0.38	0.11-2.35	No 110/54 Yes 2/2	2.00	0.49	0.60-16.99	No 258/129 Yes 10/5	1.00	1.00	0.28-14.20
Glues	No 143/69 Yes 3/4	2.67	0.20	0.59-11.91	No 111/53 Yes 1/3	6.00	0.12	0.62-57.68	No 264/127 Yes 4/7	3.50	0.05	1.02-11.96
Hair dye use	No 90/44 Yes 56/28	1.00	1.00	0.52-1.91	No 70/29 Yes 42/27	1.82	0.12	0.85-3.93	No 167/76 Yes 101/57	1.30	0.29	0.80-2.10
History of hepatitis	No 136/67 Yes 10/6	1.12	0.72	0.42-3.46	No 105/50 Yes 7/6	1.80	0.31	0.57-5.69	No 251/121 Yes 17/13	1.58	0.23	0.74-3.36
History of diabetes	No 139/72 Yes 7/1	0.22	0.18	0.02-2.01	No 102/53 Yes 10/3	0.55	0.39	0.14-2.19	No 250/130 Yes 18/4	0.38	0.10	0.12-1.22

#Number of controls/number of cases that responded either "No" or "Yes" to "ever" exposed to a specific risk factor
*****Statistically significant; p- value is less than 0.05

Table 4. Risk due to benzene exposure indices by AA subtypes

MAA[†]					SAA								
Highest BZ score	**#Num Cntl/Case**	**Odds Ratio**	**p-value**	**95% CI**	**Highest BZ score**	**#Num Cntl/Case**	**Odds Ratio**	**p-value**	**95% CI**	**Score-months[††]**	**Odds Ratio**	**p-value**	**95% CI**
2	4/2	1.0	0.99	0.15-6.50	2	1/3	6.0	0.12	0.62-58	42-378	0.67	0.70	0.82-5.41
3	6/3	1.05	0.95	0.24-4.64	3	5/4	1.71	0.46	0.41-7.05	379-505	0.44	0.41	0.05-3.05
4	2/2	2.01	0.49	0.28-14.4	4	1/3	6.0	0.12	0.62-58				

[†]Model for benzene score-months would not converge
[††]unconditional model
#Number of controls/number of cases "ever" exposed to benzene with score "2, 3 or 4"
*Statistically significant; p- value is less than 0.05

Table 5. Summary of multivariate conditional logistic regression analysis for the development of MAA

Multivariate Analysis for MAA			
Risk Factor	**Odds Ratio**	**p-value**	**95% CI**
Lived on farm	3.08	0.004*	1.43-6.62
Hair dye	1.95	0.12	0.85-4.47

*Statistically significant; p- value is less than 0.05

Table 6. Summary of multivariate conditional logistic regression analysis for the development of SAA

Multivariate Analysis for SAA			
Risk Factor	**Odds Ratio**	**p-value**	**95% CI**
Benzene	3.25	0.03*	1.12-9.44
Lived on farm	1.68	0.15	0.83-3.41
Livestock	4.59	0.08	0.83-25.38
Body mass index	0.92	0.11	0.84-1.02
Diabetes	0.09	0.07	0.006-1.23

*Statistically significant; p- value is less than 0.05

Table 7. Summary of multivariate logistic regression analysis for the development of MAA in males.

Multivariate Analysis for Males with MAA			
Risk Factor	**Odds Ratio**	**p-value**	**95% CI**
Multiple non-benzene chemical exposure	0.13	0.01*	0.02-0.64
Body mass index	1.19	0.07	0.98-1.44
Use of hair dye	11.8	0.007*	1.96-71.6

*Statistically significant; p- value is less than 0.05

Table 8. Summary of multivariate logistic regression analysis for the development of MAA in females.

Multivariate Analysis for Females with MAA			
Risk Factor	**Odds Ratio**	**p-value**	**95% CI**
Lived on farm	5.4	0.009*	1.5-19.8

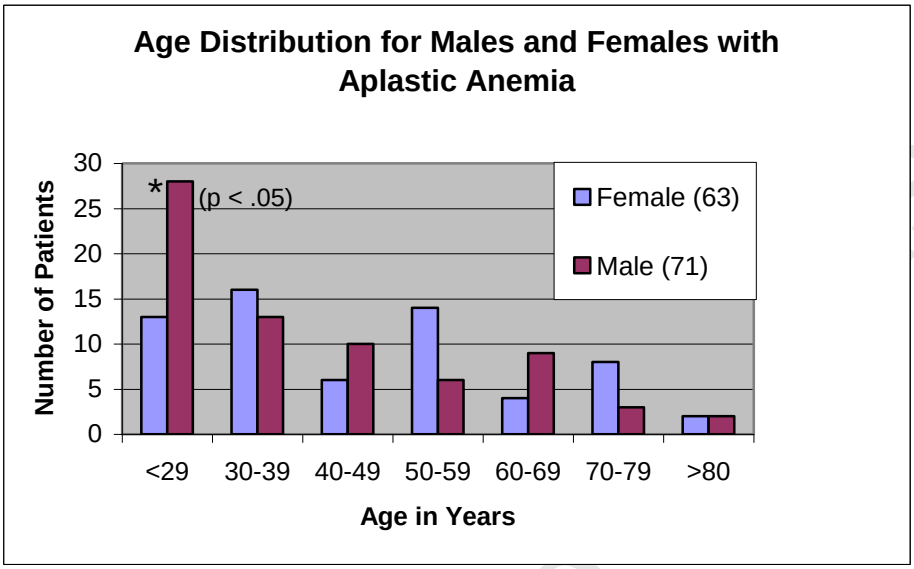
*Statistically significant; p- value is less than 0.05

Table 9. Summary of multivariate logistic regression analysis for the development of SAA in males.

Multivariate Analysis for Males with SAA			
Risk Factor	**Odds Ratio**	**p-value**	**95% CI**
Benzene	8.3	0.02*	1.42-49.3
Hepatitis	6.8	0.05	1.00-46.5
Pesticides	6.1	0.03*	1.13-33.0
Body mass index	0.8	0.16	0.74-1.05
Cutting oils	3.6	0.14	0.65-20.7

*Statistically significant; p- value is less than 0.05

Figure 1. Age and gender distribution of patients diagnosed with AA in Shanghai, China over a 4 year period. A total of 71 males and 63 males participated in this study with an age distribution at presentation of 18 to 87 years.



*Statistical significance (p- value is less than 0.05) in the numbers of males versus females presenting with AA at the age of 29 years or younger.