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Blood arsenic as a biomarker of arsenic exposure: Results from a prospective study

Marni Hall [a], Yu Chen [b], Habibul Ahsan [b,d], Vesna Slavkovich [a], Alexander van Geen [c], Faruque Parvez [a], Joseph Graziano [a,*]

[a] *Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University Medical Center, 60 Haven Avenue B1, New York, NY 10032, United States*
[b] *Department of Epidemiology, Mailman School of Public Health, Columbia University, United States*
[c] *Lamont-Doherty Earth Observatory of Columbia University, United States*
[d] *Herbert Irving Comprehensive Cancer Center, Columbia University, United States*

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Abstract

Exposure to arsenic (As)-contaminated drinking water affects millions of people worldwide. Arsenic exposure is associated with skin lesions, skin, lung, kidney and liver cancers, neurologic and cardiovascular effects. Past studies involving biomarkers of As exposure have typically examined urinary As (UAs) (adjusted for urinary creatinine), hair or toenail As, but not blood As (BAs) since blood concentrations are exceedingly low and are not detectable by conventional atomic absorption spectrophotometric techniques.

In a case-cohort analysis of 303 newly diagnosed cases of skin lesions, and 849 subcohort members randomly selected from 8092 participants in the health effects of as longitudinal study (HEALS) in Araihazar, Bangladesh, we measured blood, urine and water As concentrations, and examined their associations with each other, and with the risk for skin lesions. BAs concentrations were highly correlated with creatinine-adjusted UAs concentrations ($r = 0.85$) and with water As (WAs) ($r = 0.75$). We observed consistent dose–response relationships between the risk of skin lesions and all the measures of As exposure. Rate ratios (RRs) for skin lesions by quintile of As exposure, adjusted for age and gender, revealed that the two highest quintiles were significantly related to an increased risk of skin lesions for each measure of exposure: BAs, UAs, WAs and a time-weighted water As variable. This prospective study confirms the increased risk of skin lesions in relation to As concentrations in blood, urine and water and also establishes that BAs is a useful biomarker of As exposure in this study population.

Keywords: Arsenic; Blood biomarker; Skin lesions; Case-cohort study; Bangladesh

1. Background

Exposure to arsenic (As)-contaminated drinking water affects tens of millions people worldwide (Rahman et al., 2001). Inorganic arsenic (InAs) dissolves from naturally occurring mineral deposits into underground aquifers by processes that are not yet fully understood. Bangladesh is one of the most severely affected regions in that roughly 50% of the country's 125 million people

Abbreviations: HEALS, health effects of arsenic longitudinal study; As, arsenic; BAs, blood arsenic; ICP-MS, inductively coupled plasma mass spectrometry

* Corresponding author. Tel.: +1 212 305 1678; fax: +1 212 305 3857.
E-mail address: jg24@columbia.edu (J. Graziano).

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consume water containing As levels greater than the 10 μg/l standard set by the World Health Organization (WHO) (Caldwell et al., 2003; Chowdhury, 2004).

Arsenicosis, As toxicity due to chronic exposure, manifests itself as a variety of diseases of the dermal, cardiovascular, nervous, hepatic, hematological, endocrine and renal systems (Hughes, 2002; Nriagu, 1994; NRC, 1999). Categorized as a class I human carcinogen by WHO, As is associated with skin, lung, bladder, kidney, and liver cancers (Smith et al., 2000). Skin is a major organ of As accumulation (Nriagu, 1994; Kadono et al., 2002) and chronic exposure produces characteristic changes in pigmentation (both hyper- and hypopigmentation), followed by hyperkeratosis, and, after prolonged exposure, skin cancer. The lifetime mortality from arsenic-related internal cancers has been estimated to be doubled due to As in drinking water in Bangladeshi population, compared with historical estimates in the absence of As exposure (Chen and Ahsan, 2004).

Studies that have employed biomarkers to assess As exposure have typically involved measurement of As concentrations in urine, hair and/or toenails, but not blood. Arsenic accumulates in hair and nails, due to the element's affinity for the abundant sulfhydryl groups in keratin; thus As concentrations in these slow growing tissues are considered to be a good measure of past exposure (Klaassen, 2001). Since single doses of As are rapidly and extensively cleared from the blood via the kidney, blood As (BAs) concentrations have been considered to reflect only recent exposure (Pomroy et al., 1980). However, with chronic and continuing exposure, steady-state concentrations in blood and urine are achieved; these have the potential to serve as biomarkers of past exposure (Morton, 1994). Urinary As (UAs) has been used in many epidemiological investigations (NRC, 2001), despite the lack of validation as a biomarker, because its collection is non-invasive and it correlates reasonably well with water As (WAs) measurements (Calderon et al., 1999). However, UAs is a reflection of As excretion and not actual tissue burden (Klaassen, 2001), and, significant complexities are introduced when urine concentrations are normalized for urine creatinine (Cr) in order to adjust for hydration status (Barr et al., 2005; Gamble and Liu, 2005). We posited that with long-term exposure, BAs – which receives inputs not only from recent exogenous exposure but also from tissue compartments – may better reflect an individual's total internal As burden.

Advances in instrumentation, notably the development of inductively coupled plasma mass spectrometry (ICP-MS), have overcome the technological limitations that previously made BAs concentrations impractical for As exposure assessment. Compared to other analytical tools used in trace metal laboratories, such as graphite furnace atomic absorption spectroscopy (GFAA), ICP-MS has superior detection limits, multi-element capabilities, and high-throughput performance (B'hymer and Caruso, 2004; Michalke, 2003), thus making BAs measurements feasible for large-scale epidemiological studies. Therefore, using a case-cohort study design, we evaluated As exposure and skin lesion risk prospectively, using multiple As exposure measures, including As concentrations in water, urine and blood.

2. Methods

2.1. The health effects of arsenic longitudinal study (HEALS)

HEALS is an ongoing prospective cohort study in Araihazar, Bangladesh with the principal aim of investigating the health effects of As exposure from drinking water. It is the parent study of the work presented here and details of the study methodologies have been presented elsewhere (Ahsan et al., 2006a). Briefly, prior to subject recruitment, water samples and geographic positional system (GPS) data were collected for a set of 5966 contiguous wells in a well-defined geographic area of 25 km^2 in Araihazar, Bangladesh. Demographic information on well users was collected in order to create a sampling frame for HEALS (Parvez et al., 2006). Between October 2000 and May 2002, 11,746 men and women aged 18 years and above were recruited, with a participation rate of 97.5%. The HEALS cohort is being followed with in-person visits at 2-year intervals. Verbal consent was obtained from each eligible respondent who agreed to participate in the study. The study procedures were approved by the Columbia University Institutional Review Board and the Ethical Committee of the Bangladesh Medical Research Council.

At recruitment, baseline whole venous blood samples were collected in 3 ml EDTA Vacutainers for 91.8% of the overall 11,746 cohort participants. A spot urine sample was collected in 50 ml acid-washed tubes at baseline and at the 2-year follow-up visit for 95.6% and 94.5% of the cohort participants, respectively. Both blood and urine samples were kept in portable coolers (carried by the research team) immediately after collection. Within 2–8 h, blood and urine samples were frozen at −20 °C in the study office in Dhaka city. All samples were kept frozen and shipped to Columbia University on dry ice within 1–2 months.

2.2. Selection of cases and subcohort

Trained physicians completed a comprehensive physical examination at the baseline and follow-up visits. Presence/absence, type, size, shape of skin lesions and extent of skin involvement were recorded. Physicians were blind to participants' As exposure information. In the present study, presence

of skin lesions was defined as the presence of any melanosis, leucomelanosis, and/or keratosis.

A case-cohort study design (Prentice, 1986) was used to evaluate the relationship between BAs levels and risk of skin lesions. Among the 9727 participants who gave both urine and blood samples and completed the physical examination at baseline, 712 had prevalent skin lesions. Excluded from the study were 923 randomly selected subjects whose blood samples were previously consumed in a study of genetic susceptibility. The present analysis included a 10.5% random sample of the remaining 8092 participants ($n = 849$) and 303 cases of newly diagnosed skin lesions. The 303 cases of skin lesions were diagnosed at the 2-year follow-up from the 8092 participants between November 2002 and April 2004. Among the 303 newly diagnosed cases, 31 were also part of the 849 subcohort members.

2.3. Measurements of As exposure

At baseline, water samples from all 5966 tube wells in the study area were collected in 50 ml acid-washed tubes following well pumping for 5 min. Total As concentration was determined by GFAA with a Hitachi Z-8200 system at the Lamont-Doherty Earth observatory of Columbia University (van Geen et al., 2002); the detection limit of the method is 5 μg/l. Samples that fell below 5 μg/l were subsequently analyzed by ICP-MS, which has a detection limit of 0.1 μg/l (Cheng et al., 2004). Analyses of time-series samples collected from 20 tube wells in the study area showed that the standard deviation of groundwater As concentration was <10 μg/l over 3 years (Cheng et al., 2005). Given that the As concentration in well water is relatively stable over time, we derived a time-weighted As measure (TWA) as a function of drinking durations and well As concentrations [TWA in μg/l = $\sum C_i T_i / \sum T_i$, where C_i and T_i denote the well As concentration and drinking duration for the ith well] (Ahsan et al., 2006b). In addition to information on the current well, drinking duration and well As concentration of the prior well were taken into account in the calculation of TWA for participants whose previous drinking well was one of our tested wells. The TWA represents the average As exposure accrued for 9 years on average in the cohort members prior to the time of baseline visits.

Total UAs concentration was measured by GFAA, using the a Perkin-Elmer AAnalyst 600 graphite furnace system, as previously described (Nixon et al., 1991). Urinary creatinine was assayed by a colorimetric Sigma Diagnostics Kit (Sigma, St. Louis, MO) in order to adjust for hydration status.

2.4. Measurement of As in whole blood

Whole blood samples were analyzed for BAs concentrations using a Perkin-Elmer Elan DRC II ICP-MS equipped with an AS 93+ autosampler. ICP-MS-DRC methods for metals in whole blood were developed from published methods (Stroh, 1993), with modifications to the blood sample preparation as suggested by the Laboratory for ICP-MS Comparison Program, Institut National de Sante Publique du Quebec. Whole blood samples were thawed, thoroughly mixed, then diluted 50 times with diluent containing 1% HNO_3 + 0.2% Triton X-100 + 0.5% NH_4OH. Samples were then centrifuged for 10 min at 3500 rpm, and the supernatant reserved for analysis. Standard solutions were used for instrument calibration. The As concentrations of standard solutions were chosen to cover the expected range of As concentrations in the blood samples: 5, 25, and 50 μg/l. Matrix-induced interferences were corrected by selection of an internal standard matched to the mass and ionization properties of the As, i.e., iridium (Ir). Stock internal standard spiking solution was prepared and added to all calibrators and samples in the same concentration, 10 ng Ir per tube. Polyatomic interferences were suppressed with the instrument's dynamic reaction cell (DRC) technology feature, utilizing oxygen as a second gas. After the instrument was calibrated, quality control samples (blood samples with known analyte concentrations obtained from the Laboratory for ICP-MS Comparison Program in Quebec) were run. Quality control blood samples were purchased to cover the range of concentrations of analytes of interest and were run each day. During the period in which all samples of this study were analyzed, the intraprecision coefficient of variation for blood As was 12.8%.

2.5. Statistical analysis

The subcohort is a good representation of the underlying source population. Spearman correlations were calculated for pairwise comparisons of BAs, WAs, and total UAs measured at baseline in the subcohort. We also performed linear regression models to evaluate the associations of WAs with BAs and total UAs controlling for age, gender, body mass index (BMI), and smoking status.

Incidence rate ratios (RRs) for skin lesions were estimated using Cox proportional hazards models with the PROC PHREG procedure of SAS. Standard errors were estimated using the robust variance estimator proposed by Barlow (Barlow et al., 1999). The random cohort was weighted by the inverse of the sampling fraction from the source population. Risk sets were created with age at the time of follow-up visit as a matching variable. For each case, members of the random subcohort whose age at the time of follow-up were older than that of the case by ≤3 years were included as the comparison for the case, i.e., those who had not been diagnosed with skin lesions at the age the case was diagnosed. Arsenic exposure categories were determined according to quintile values in the subcohort. Besides age and gender, RRs were additionally adjusted for BMI and smoking status.

3. Results

Table 1 summarizes the characteristics of the skin lesion cases ($n = 303$) and the subcohort ($n = 849$). Cases were more likely to be male, older, less educated and to have ever smoked. BMI was essentially the same in

Table 1
Characteristics of the 849 subcohort members and 303 skin lesion cases identified at follow-up from the HEALS

Baseline characteristics	Subcohort ($n = 849$)	Skin lesion cases identified at follow-up ($n = 303$)
Gender		
Male (%)	37.0	70.3
Mean age (years)	36.6	45.0
Mean BMI	19.9	19.4
Unknown BMI (*N*)	7	4
Mean education level (years)	3.7	2.9
Cigarettes smoking status		
Ever-smokers in men (%)	70.7	81.7
Ever-smokers in women (%)	5.6	11.1
Baseline As exposure variables		
Drinking duration of baseline well (years)	6.9	8.0
Total urinary As (μg/l)	137.3	172.0
Urinary creatinine (g/l)	58.1	60.6
Blood As (μg/l)	10.8	14.3
Baseline well As (μg/l)	103.1	157.4
Drinking duration with known As levels (years)	8.6	10.1
Time-weighted well As (μg/l)	101.8	147.4
Unknown time-weighted well As	36	18

both groups. For each measure of As exposure, the mean baseline value was higher in cases; these included UAs, BAs and WAs, and TWA. Compared to the subcohort, cases were also more likely to have switched their source of drinking water since the baseline assessment 2 years earlier.

Rate ratios for skin lesions by quintiles of the four As exposure measures are shown in Table 2. After adjustment for age and gender, there was a dose–response trend for risk of skin lesions in relation to all four As measures. Rate ratios were similar after additional adjustments for BMI and smoking status.

Among subcohort members, BAs was strongly correlated with WAs and with UAs. Plots of the relationships between WAs, BAs and UAs of subcohort members who drank from only one well are shown in Fig. 1, for the entire range of exposures ($n = 724$), and for WAs < 50 μg/l ($n = 341$). In each case, the strongest correlations were observed between BAs and UAs ($r = 0.85$ and 0.65, respectively).

Multiple regression analyses revealed that BAs and UAs were positively associated with WAs concentration and smoking, and inversely related to BMI (Table 3). In comparison to women, men had significantly lower UAs concentrations, both before and after adjustment for creatinine. Men also had higher BAs levels, though this did not quite achieve significance. BAs also appeared to decrease with age.

4. Discussion

Here, we report findings from a case-cohort analysis regarding the risk of skin lesions in relation to four measures of As exposure: well water, urine, blood and time-weighted As intake. We observed a dose–response relationship between the risk of skin lesions and all four exposure measures. BAs and UAs were most strongly correlated with one another and were similarly correlated with WAs. Bioavailability of arsenic in foods is unknown but may partly contribute to the imperfect correlations between biomarkers of arsenic and well arsenic concentration. Arsenic content in foodstuffs depends on the arsenic concentration in the soil, in the water used for washing and cooking, in the water used for irrigation, in pesticides, and in different cooking processes (Roychowdhury et al., 2002). Therefore, evaluation of arsenic content in diet is complex and beyond the scope of the current study but will be the subject of future HEALS investigations.

In addition, we evaluated the strength of the relationship between these biomarkers and As-induced skin lesions. While several previous studies investigated the risk of As-related skin lesions using either ecological or retrospective measures (Ahsan et al., 2000; Chen et al., 1988; Haque et al., 2003; Tseng, 1977) this is the first large prospective investigation using individual-level exposure measures.

We observed increased RRs for skin lesions in relation to all As exposure measures. The dose–response associations observed in the present study confirm our findings in the cross-sectional analysis of the baseline data, but are somewhat weaker than those observed in the baseline cross-sectional study of the entire cohort of 11,746 subjects (Ahsan et al., 2006b). Statistical significance was achieved only in the highest two quintiles of As exposure. This is consistent with a tendency for exposure-disease associations to be weaker in prospective cohort analyses as compared to cross-sectional or case–control analyses (Rothman, 1998). Alternatively, the changes in As exposure during the follow-up period may have influenced the rate ratio estimates, especially those associated with low-level As exposure. However, in a separate analysis, OR estimates with additional adjustment of UAs measured at the time of follow-up remained virtually the same (data not shown). In future

Table 2
Adjusted rate ratios for skin lesions by different measures of as exposure

Measures of As exposure (quintile)	Mean level	Total no.	No. of subcohort	No. of cases	Rate ratios[a] (95% CI)	Rate ratios[b] (95% CI)
Baseline water As (μg/l)						
0.1–7	2.4	228	184	48	1.00	1.00
8–38	22.3	185	156	31	0.88 (0.49–1.58)	0.92 (0.50–1.67)
39–94	65.1	217	173	48	1.20 (0.69–2.08)	1.27 (0.73–2.20)
95–189	137.6	243	171	81	1.90 (1.14–3.17)	1.92 (1.14–3.24)
190–564	311.7	248	165	95	2.38 (1.44–3.92)	2.50 (1.52–4.14)
Baseline total urinary As (μg/l)						
3–35	21.9	207	175	36	1.00	1.00
36–64	50.2	215	166	54	1.72 (0.98–3.02)	1.63 (0.92–2.89)
65–113	86.6	214	169	54	1.67 (0.96–2.92)	1.73 (0.99–3.02)
114–201	155.6	235	172	68	1.97 (1.11–3.47)	2.00 (1.13–3.56)
202–1230	368.5	250	167	91	2.97 (1.63–5.39)	3.16 (1.73–5.76)
Baseline blood As (μg/l)						
1.6–5.4	4.0	208	173	41	1.00	1.00
5.5–7.5	6.6	210	173	40	1.18 (0.76–1.84)	1.22 (0.70–2.12)
7.6–10.4	8.9	212	165	51	1.21 (0.79–1.84)	1.21 (0.69–2.13)
10.5–15.0	12.4	235	172	70	1.60 (1.08–2.38)	1.68 (0.99–2.86)
15.1–63.9	23.1	256	166	101	2.43 (1.67–3.54)	2.54 (1.51–4.27)
Time-weighted As (μg/l[*])						
0.1–7.9	2.5	199	163	40	1.00	1.00
8.0–41	24.0	196	163	35	1.09 (0.60–2.00)	1.14 (0.61–2.11)
42–94	66.1	210	164	51	1.38 (0.78–2.45)	1.44 (0.81–2.58)
95–175	131.7	212	161	58	1.63 (0.94–2.83)	1.66 (0.94–2.93)
176–564	288.9	252	162	101	2.67 (1.57–4.56)	2.85 (1.66–4.89)

[*] A total of 52 participants with unknown information on time-weighted average As were excluded from the analysis.
[a] Rate ratios were adjusted for age and gender. RRs in relation to total urinary As were adjusted for urinary creatinine additionally.
[b] Rate ratios were adjusted for age, gender, BMI, and smoking status. Eleven subjects with unknown BMI were excluded from the analysis.

analyses of this cohort, with increases in follow-up time and the number of cases accrued, we will be able to evaluate whether certain subgroups of the population are more susceptible to the risk of skin lesions at low-levels of As exposure. This will be noteworthy since the risk of As-related disease at the current maximum containment level (MCL) has not been prospectively measured, but instead relies upon extrapolations from high-dose scenarios (Schoen et al., 2004; NRC, 1999). In addition, we will be able to evaluate whether long-term switching to safe water has an influence on the dose–response relationship.

Table 3
Relationships[a] of well As and other characteristics with blood As and urinary As in the subcohort

	Blood As (μg/l)			Urinary As (μg/l)[b]			Urinary creatinine-adjusted As (μg/g creatinine)		
	Parameter estimate	Standard error	*p*-Value	Parameter estimate	Standard error	*p*-Value	Parameter estimate	Standard error	*p*-Value
Male	0.971	0.618	0.117	−25.854	11.650	0.027	−59.374	22.947	0.010
Water As (μg/l)	0.034	0.002	<0.001	0.601	0.034	<0.001	1.157	0.068	<0.001
BMI	−0.224	0.064	0.001	−4.632	1.217	0.000	−11.352	2.389	<0.001
Age (years)	−0.046	0.024	0.052	−0.685	0.441	0.121	−0.994	0.874	0.256
Smoking status									
Ex-smokers	−0.934	0.999	0.350	1.300	18.698	0.945	−35.762	37.093	0.335
Current, light smokers	1.759	0.763	0.021	23.643	14.271	0.098	20.400	28.316	0.471
Current, heavy smokers	1.770	0.916	0.054	20.891	17.141	0.223	42.549	34.011	0.211

[a] Estimated from multiple linear regression models.
[b] Urinary creatinine was additionally controlled in the model.

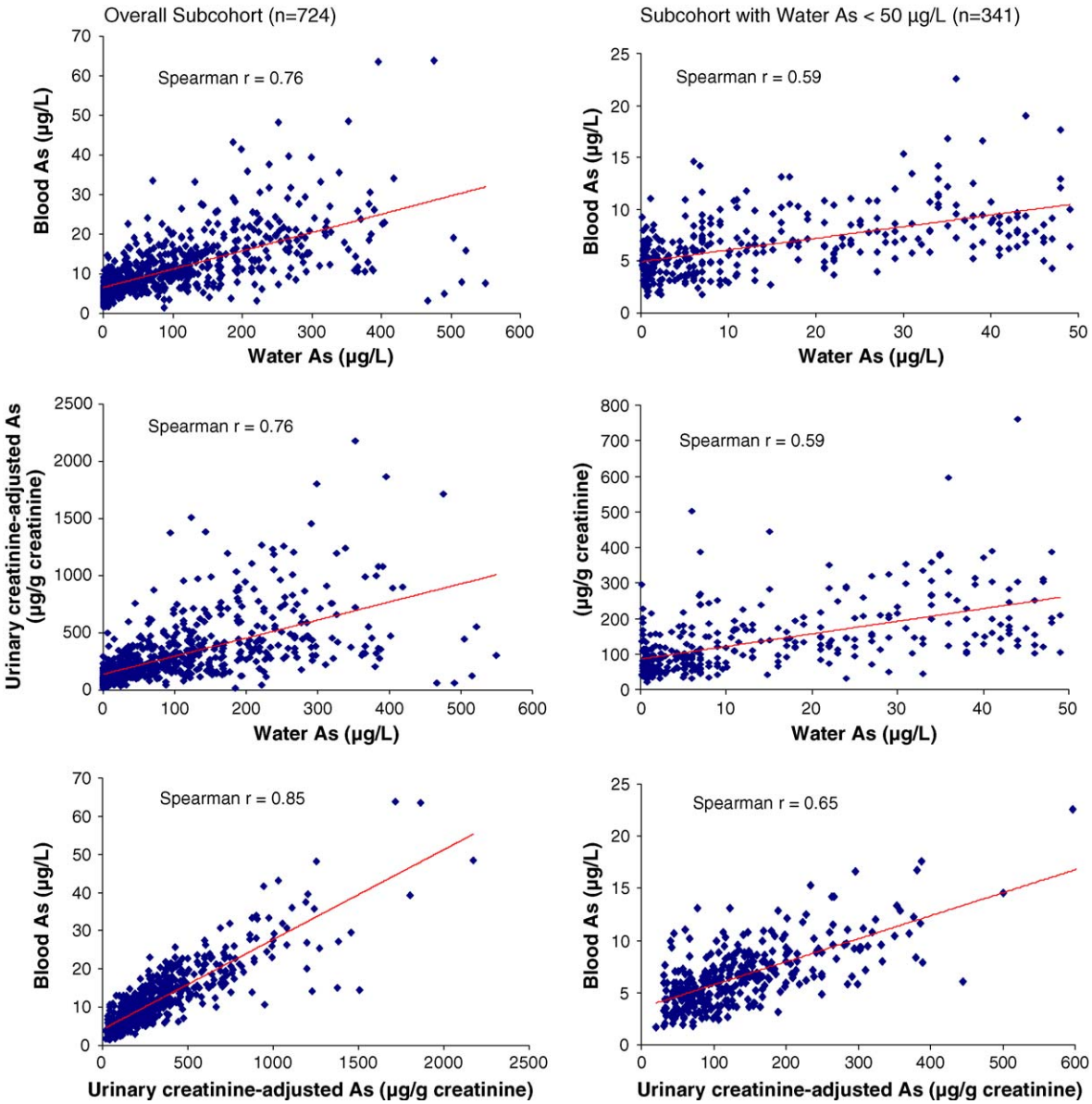

Fig. 1. Relationships between WAs, BAs and UAs exposure measures at baseline: Spearman correlations for pairwise comparisons of BAs and WAs, UAs and WAs and BAs and UAs are shown in the top, middle and bottom rows, respectively. The left hand column are subcohort members that drank from one well only ($n = 724$), the right hand column are subcohort members with WAs < 50 µg/l ($n = 341$).

Better biomarkers are needed to help elucidate mechanistic knowledge of As toxicity and for risk assessment (Tchounwou et al., 1999; Schmitt et al., 2005). In applying The National Academy of Science risk assessment/management paradigm to As toxicity, Tchounwou et al. described the need for better dose–response assessment, exposure assessment and risk characterization, in order to realistically make risk management decisions (Tchounwou et al., 2004). Total BAs levels can contribute to these efforts because they represent a measure of internal dose. BAs metabolite data may provide insight into metabolic differences that lead to differences in susceptibility to arsenicosis but, because BAs metabolites are non-detectable in the low exposure range, they have limited utility in risk assessment studies. Accordingly, dose–response studies of individuals with ongoing chronic exposure, over a wide range of exposures are best served by analyzing total BAs concentrations. Comparative analyses of BAs levels with biomarkers of early biologic effects due to As

toxicity, such as DNA damage or markers of oxidative stress, will likely shed light on the biological variations that render some more vulnerable. Indeed, using atomic absorption spectrophotometry Wu et al. reported positive associations between BAs and plasma reactive oxidants, and an inverse relationship with plasma antioxidant capacity (Wu et al., 2001). In addition, using cDNA microarray technology, they determined that inflammatory mediator genes, thought to play a role in As-induced atherosclerosis, increased as BAs levels increased (Wu et al., 2003). It therefore appears promising that the use of BAs as an exposure biomarker may reveal early biological effects that precede overt disease.

Another major advantage of BAs measurements is that, unlike UAs, adjustments for creatinine are not necessary. Several demographic variables such as age, sex, race/ethnicity, and BMI are significant predictors of urinary creatinine (Barr et al., 2005), thus confounding the expression of UAs as μg/g creatinine. In addition, folate is a critical driver of As methylation reactions. Gamble and co-workers reported that expression of total UAs per gram creatinine confounds relationships between folate and As metabolism since muscle creatine – the precursor of creatinine – is the primary product of one-carbon metabolism, a folate-dependent pathway (Gamble and Liu, 2005; Gamble et al., 2005). Moreover, urinary creatinine is a significant determinant of the proportion of UAs that is eliminated as dimethylarsenic (DMA), likely because urinary creatinine indirectly reflects the integrity and/or sufficiency of the one-carbon metabolic pathway. Elimination of the complexities introduced by adjustment of any urinary analyte for creatinine appears to offer a major advantage to the use of blood level as a biomarker.

As in our previous baseline cross-sectional analyses, we found that male participants, smokers of tobacco products, and participants with a low BMI were more susceptible to As-related skin lesions at a given level of As exposure (Ahsan et al., 2006b; Chen et al., 2006). It is interesting to note that in the current study, male gender was positively related to BAs and inversely associated with UAs, and that BAs was more strongly associated with smoking status than UAs. Undoubtedly, because UAs measurements are non-invasive, they will likely continue to be an important tool in biomonitoring exposed populations, despite the fact that they involve two sets of measurements (i.e., As and either creatinine or specific gravity), and two sources of experimental error.

Toenail analysis provides a reliable measure of past As exposure (Karagas et al., 2000). However, nail As reflects several months of exposure but not recent exposure, and it cannot be used to assess relationships between exposure and labile by-products of metabolism (Wu et al., 2001). Current methodologies employed to analyze nail As measure total As only. In addition, nail As may have limited utility in highly exposed populations. In a study of nails as a biomarker of As exposure in Inner Mongolia, Schmitt et al. reported that well water As concentrations in the high exposure group were 50-fold greater than the low exposure group, but nail As concentrations showed only a 20-fold difference, perhaps suggesting that nails can become saturated with As (Schmitt et al., 2005). Accordingly, this biomarker may be most appropriate when individual water As exposure is very low or when a time-integrated exposure measure is desired.

In summary, using multiple As exposure measures, we found a dose–response relationship between the risk of skin lesions and As exposure from drinking water, confirming the findings of numerous cross-sectional studies in the literature (Tondel et al., 1999; Haque et al., 2003; Rahman et al., 1999, 2001; Tseng, 2002; Guo et al., 2001; Mitra et al., 2002; Ahsan et al., 2006b). We also demonstrated that BAs is a good biomarker of As exposure in this population. The strong correlations observed between BAs and UAs, and between BAs and water As validates the use of all three of biomarkers in future HEALS studies, where the appropriate choice of an exposure marker may be based on study design and logistical parameters.

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