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# Development and validation of a fluoride bone injury risk prediction model

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**Key words:** Fluoride; fluoride bone injury; risk prediction model

**Parole chiave:** Fluoruro; lesioni ossee da fluoruro; modello di predizione del rischio

**Summary**

**Background:** *Fluoride bone injury affects millions of people exposed to fluoride worldwide, and has no treatment - prevention is the only solution.* **Objectives:** *A risk prediction model was developed to identify workers at high risk for fluoride bone injury in aluminum production.* **Methods:** *We collected data from the Molecular Epidemiology Study of Fluoride Bone Injury. 120 fluoride bone injury cases and 120 controls were involved in the study. Logistic regression was used to determine variables in the risk prediction model. Predictive accuracy was validated with bootstrap method. Potential risk cut-offs was evaluated with receiver operating characteristic curve.* **Results:** *Working history, urinary fluoride, osteocalcin, bone alkaline phosphatase and calcitonin receptor gene polymorphism were included in the final prediction model. The model had very good calibration and discrimination (C index=0.986; Brier score 0.014).* **Conclusions:** *Our fluoride bone injury risk prediction model performed well in the present data, and the working history, urinary fluoride, osteocalcin, bone alkaline phosphatase, and calcitonin receptor gene polymorphism were identified as predictors. The model could be used to assess the fluoride bone injury risk, and identify the susceptible workers.*

**Riassunto**

***«Sviluppo e validazione di un modello di predizione del rischio di lesioni ossee da fluoruro»***. **Introduzione:** *Le lesioni ossee da fluoruro colpiscono milioni di persone esposte a fluoruro in tutto il mondo. Non esiste cura, la prevenzione è l'unica soluzione.* **Obiettivi:** *Sviluppare un modello di predizione del rischio per identificare i lavoratori ad alto rischio di lesioni ossee da esposizione a fluoruro nel settore della produzione dell'alluminio.* **Metodi:** *Sono stati raccolti dati dallo Studio epidemiologico molecolare sulle lesioni ossee da fluoruro. Lo studio ha riguardato 120 casi di lesioni ossee da fluoruro e 120 controlli. È stata usata la regressione logistica per determinare le variabili da inserire nel modello di predizione del rischio. L'accuratezza della predizione è stata validata con il metodo Bootstrap. Il cut-off del rischio potenziale è stato valutato con una curva Roc (receiver operating characteristic).* **Risultati:** *Storia lavorativa, fluoruro urinario, osteocalcina, fosfatasi alcalina ossea e polimorfismo del gene del recettore della calcitonina sono stati inclusi nel modello predittivo finale. Il modello mostra una calibratura e una discriminazione molto buone (C index=0.986; Brier score 0.014).* **Conclusioni:** *Il modello di predizione delle lesioni ossee da fluoruro ha*

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*funzionato bene con i dati a disposizione. Storia lavorativa, fluoruro urinario, osteocalcina, fosfatasi alcalina ossea e polimorfismo del gene del recettore della calcitonina sono stati identificati come predittori. Il modello potrebbe essere utilizzato per valutare il rischio di lesioni ossee da fluoruro e per identificare i lavoratori suscettibili di sviluppare la patologia.*

## Introduction

Fluorine a powerful oxidizing agent, is used increasingly in a variety of industries. Due to rapid industrialization, health problems among industrial workers due to fluoride poisoning are on the rise. Emission of fluoride dust and fumes from the smelters of primary aluminum producing industries is dissipated in the work environment and poses occupational health hazards. Use of cryolite as a flux in the conversion of alumina to aluminum, is the major source for fluoride emission (9). Fluoride stimulates the osteogenetic process, resulting in an increase in bone mass (2). However, chronic ingestion of high dose of fluoride can lead to fluoride bone injury (6). Fluoride bone injury is very common where there is industrial exposure to fluoride from dust or fumes (14). Fluoride bone injury is especially prevalent in parts of China, India, and Africa and affects millions of people worldwide (1, 7, 13). Fluoride bone injury often results in osteosclerosis of the skeleton with significant long-term difficulties, including impaired neck and lumbar mobility, aching of the axial skeleton, kyphosis, and painful lower extremities, ultimately causing crippling and incapacitation (10). Fluoride bone injury has no treatment - prevention is the only solution.

Risk prediction model has been used in the study of chronic and infectious diseases to identify important risk factors and to quantify or rank each factor's comparative importance in the development of disease (8, 11). Unlike risk factor studies, which identify characteristics that increase a patient's risk for disease, risk prediction model allows researchers to "score" patients based on the importance of these characteristics, thereby identifying patients at highest risk for disease. If applied to fluoride bone injury prevention in workers exposed to fluoride, risk prediction model potentially could allow occupational physicians to identify workers at highest risk for fluoride bone injury and intervene before they become ill. Risk prediction model of fluoride bone injury is not well studied.

To our knowledge, no study has used this method to identify workers at high-risk for fluoride bone injury. The purpose of this study was to develop and validate a fluoride bone injury risk prediction model that could ultimately be employed real-time to prevent fluoride bone injury cases in aluminum plant workers.

## Methods

We used data from participants in the Molecular Epidemiology Study of Fluoride Bone Injury, a project sponsored by the China National Nature Science Foundation in Wuhan, China. Data collected included age, smoking status, alcohol drinking, working history, pulse, blood pressure, urinary fluoride, osteocalcin, calcitonin, bone alkaline phosphatase, and calcitonin receptor gene polymorphism.

The study population has been described in full previously (12). In brief, eligible cases for our study were smelter workers of aluminum plant aged 20-56 years, and were diagnosed as fluoride bone injury in occupational physical examinations. 120 cases were enrolled in this study according to Chinese Diagnostic Criteria of Industrial Fluorosis, combined with fluoride exposure history (more than 5 years) and the clinical symptoms, ruled out the diseases that had similar X-ray changes. 120 controls were recruited from occupational physical examinations in the same aluminum plants. Controls were selected by frequency matching with cases on age, working category, smoking, and alcohol consumption. For the purposes of developing risk prediction models to discriminate patients with fluoride bone injury from workers without fluoride bone injury, the present analyses compared the fluoride bone in-

jury cases to the smelter worker controls only. Due to the lack of female workers, all participants in our study were male. Written informed consent was obtained from each subject. The research was approved by the Institutional Ethical Boards of Tongji Medical College, Wuhan China, and had been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Data from this Molecular Epidemiology Study were used to develop a fluoride bone injury risk prediction model. The predictors were identified using forward stepwise logistic regression. We included in the risk model those variables that were statistically significantly associated with fluoride bone injury at the 5% level in univariate analyses and we performed a forward stepwise regression procedure, whereby those factors losing their significance in the analysis were dropped. Finally, a set of variables that best predicted the risk of fluoride bone injury were determined.

The accuracy of the model was assessed using tests for discrimination (C statistic) and calibration (Brier score) (5). The C statistic, also known as the area under the curve (AUC), measures discrimination: the ability of the model to separate cases from non-cases. The closer the C-statistic is to 1, the better the discrimination of the model. The Brier score measures calibration: the closeness of predicted probabilities from the risk model to the observed outcome (4). The closer the Brier score is to 0, the better the calibration of the model. The final model was bootstrapped 500 times to validate predictive accuracy (3). Briefly, the bootstrapping methods involved drawing a sample from the original data and estimating the bootstrap regression coefficients using the bootstrap sample. Next, the apparent predictive accuracy index was calculated by applying coefficients to the bootstrap sample, and the predictive accuracy index was calculated by applying the coefficients to the original data. The bootstrap optimism was calculated, and the process was repeated 500 times. Finally, the average optimism was subtracted from the apparent predictive accuracy index to obtain the bias-corrected predictive accuracy index. A receiver operating characteristic (ROC) curve was created to assess which fluoride bone injury risk cut-offs might be used to initiate an intervention. Statistical analyses were performed with SPSS (Chicago, IL), and R (Vienna, Austria).

## Results

The final dataset included 240 admissions with 120 fluoride bone injury cases. All the potential predictive covariates with their univariate analyses are presented in table 1. In the univariate analyses among cases and controls, working history, urinary fluoride, osteocalcin, calcitonin, bone alkaline phosphatase, and calcitonin receptor gene polymorphism were all statistically significantly associated with fluoride bone injury risk.

The variables retained in the final logistic regression model included working history, urinary fluoride, osteocalcin, bone alkaline phosphatase, and calcitonin receptor gene polymorphism (table 2). Because there were relatively few persons with TC and TT genotypes of calcitonin receptor gene, TC and TT genotypes were combined into a single category in the logistic regression analyses.

The risk prediction model for fluoride bone injury had good discrimination, with an AUC of 0.986 (95%CI 0.969-1.004), and demonstrated excellent calibration in the original dataset and the bootstrap samples (Brier score=0.014) (figure 1).

Table 3 presents the predicted probabilities of developing fluoride bone injury with the sensitivity and specificity of the risk prediction model at different thresholds of fluoride bone injury risk. For example, at the 0.509 level, the predicted probability of fluoride bone injury is 50.9%, the sensitivity of the model is 95.0% and the specificity is 97.5%. As the predicted probability of developing fluoride bone injury increases, the sensitivity of the model decreases and the specificity increases.

Figure 2 presents the ROC curve, graphically depicting the ability of the model to discriminate between true cases and true non-cases. Based on these data, potential thresholds for intervention could be when the probability of developing fluoride bone injury reaches 0.649 (64.9% risk of Fluoride bone injury). At the 0.649 level, the number of fluoride bone injury cases would be up to 113 (94.2% of the total fluoride bone injury cases included in this

**Table 1** - Univariate analyses of collected variables among fluoride bone injury cases and controls

| Variable | Case | Controls | *P* |
|---|---|---|---|
| Age, years, Mean±SD | 36.51±5.73 | 37.02±4.51 | 0.439 |
| Working history, years, Mean±SD | 15.78±6.21 | 10.35±4.63 | 0.000 |
| Pulse, times/min, Mean±SD | 70.68±6.68 | 70.34±7.18 | 0.703 |
| Systolic pressure, mmHg, Mean±SD | 122.29±13.31 | 120.47±11.48 | 0.257 |
| Diastolic pressure, mmHg, Mean±SD | 83.95±9.80 | 82.02±9.32 | 0.120 |
| Urinary fluoride, mg/L, Mean±SD | 6.58±5.39 | 3.04±2.53 | 0.000 |
| Osteocalcin, μg/L, Mean±SD | 29.67±6.60 | 19.13±5.67 | 0.000 |
| Calcitonin, pg/ml, Mean±SD | 8.31±1.82 | 6.04±1.41 | 0.000 |
| Bone alkaline phosphatase, μg/L, Mean±SD | 32.12±5.06 | 20.60±2.66 | 0.000 |
| Smoking status, n. (%) | | | |
| Never smoker | 39 (32.5) | 45 (37.5) | |
| Ex-smoker | 11 (9.2) | 11 (9.2) | |
| Current smoker | 70 (58.3) | 64 (53.3) | 0.706 |
| Alcohol drinking, n. (%) | | | |
| Non-drinker | 16 (13.3) | 16 (13.3) | |
| Occasional drinker | 63 (52.5) | 58 (48.4) | |
| Frequent drinker | 41 (34.2) | 46 (38.3) | 0.781 |
| Calcitonin receptor gene polymorphism, n. (%) | | | |
| CC | 78 (65.0) | 98 (81.7) | |
| TC | 33 (27.5) | 18 (15.0) | |
| TT | 9 (7.5) | 4 (3.3) | 0.014 |

**Table 2** - Variables in final risk prediction model

| Variable | OR | 95%CI |
|---|---|---|
| Working history | 1.232 | 1.078-1.408 |
| Urinary fluoride | 1.351 | 1.101-1.659 |
| Osteocalcin | 1.172 | 1.055-1.302 |
| Bone alkaline phosphatase | 1.788 | 1.448-2.207 |
| Calcitonin receptor gene polymorphism (TC&TT genotypes) | 8.725 | 1.477-51.529 |

study) and the number of patients identified as high risk for fluoride bone injury who did not develop the disease would be 127.

## Discussion

In this study we developed a fluoride bone injury risk prediction model based on existing data from our ongoing project. We used a rigorous statistical approach to determine the most important panel of risk factors to predict the fluoride bone injury in smelter workers of aluminum plant. The risk assessment provided by our model was strong according to statistical measures that evaluate model discrimination and calibration. The discrimination of our model (AUC=0.986) was high, which indicates the model successfully identified workers who would develop Fluoride bone injury. Calibration was low (Brier score=0.014), suggesting the risk prediction model can accurately estimate the future probability of the event in question (i.e., a worker developing fluoride bone injury).

Preventing damage or disease caused by occupational hazards is the purpose of occupational health. To prevent fluoride bone injury, improving the occupational hazards control measures is important, meanwhile identifying the susceptible workers is necessary. Fluoride bone injury often results in osteosclerosis of the skeleton with significant long-term difficulties, including impaired neck and lumbar mobility, aching of the axial skeleton, kyphosis, and painful lower extremities, ultimately causing crippling and incapacitation. The occurrence of fluoride

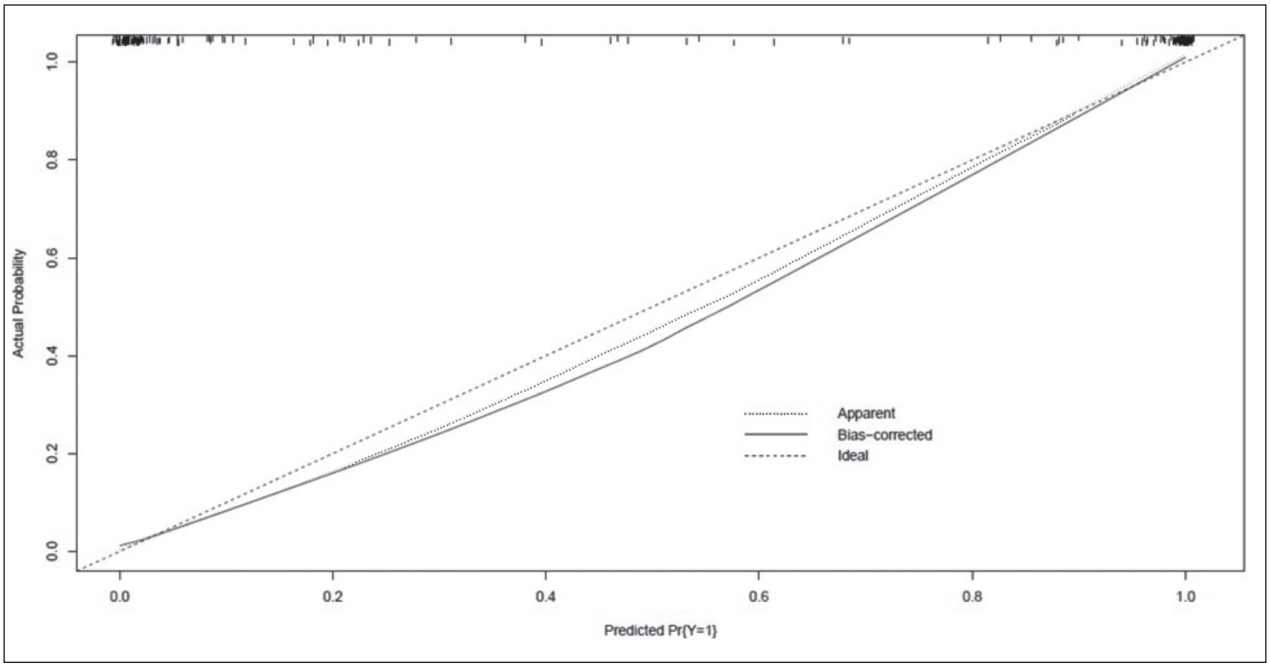


**Figure 1** - Fluoride bone injury calibration plot. Key: The ideal line represents a scenario when all cases are predicted by the model. The apparent is the performance of the model on the derivation cohort. The bias-corrected is the performance on the model after boot-strapping

**Table 3** - Comparison of fluoride bone injury risk prediction model predicted probability, sensitivity, and specificity at different levels of fluoride bone injury risk

| Predicted probability of developing fluoride bone injury | Sensitivity | Specificity |
|---|---|---|
| 0.392 | 0.967 | 0.958 |
| 0.478 | 0.958 | 0.975 |
| 0.509 | 0.95 | 0.975 |
| 0.595 | 0.942 | 0.992 |
| 0.649 | 0.942 | 1 |
| 0.866 | 0.9 | 1 |
| 0.960 | 0.85 | 1 |
| 0.968 | 0.8 | 1 |

bone injury is a complex process that involves both exogenous and endogenous factors. According to our previous study, working history, urinary fluoride, and TC&TT genotype were the main risk factors of fluoride bone injury in aluminum plant workers. Working history in our study means the duration of work exposed to fluoride. Obiviously, the longer the length of working history, the higher fluoride bone

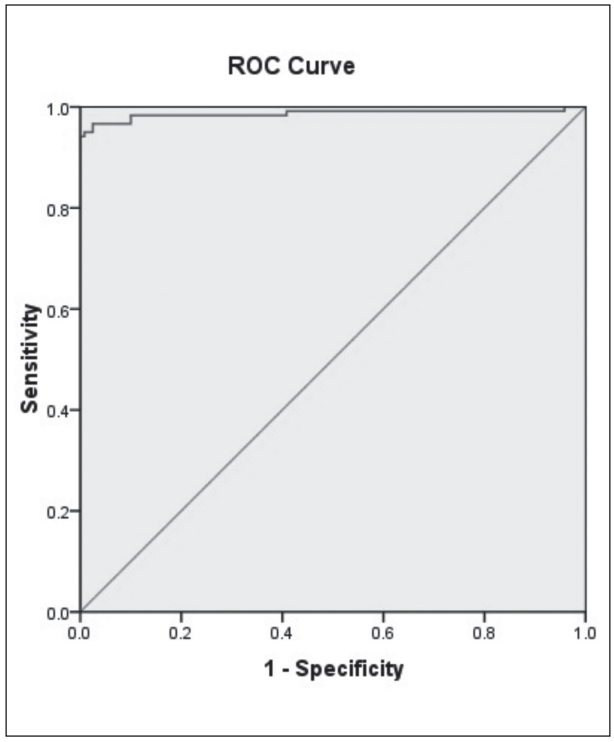


**Figure 2** - ROC curve for Fluoride bone injury risk prediction model

injury risk. Fluoride exposure leads to high fluoride burden, which can finally result in fluoride bone injury. Urinary fluoride is stable enough to reflect the fluoride burden. Workers with TC&TT genotype are more susceptible to fluoride bone injury (12). These factors could lead to the accumulation of fluorapatite in bone. Fluorapatite is difficult to be absorbed, could destroy the dynamic balance of bone resorption and formation, and leads to bone metabolic disorders (the early change of Fluoride bone injury). Bone metabolism indicators mainly include osteocalcin, calcitonin, and bone alkaline phosphatase. Osteocalcin and bone alkaline phosphatase reflect osteoblast activity directly, and calcitonin can inhibit osteoclast activity. So we selected the main risk factors and metabolism indicators as candidate predictor variables to develop the risk prediction model.

The final risk model included terms for working history, urinary fluoride, osteocalcin, bone alkaline phosphatase, and calcitonin receptor gene polymorphism. Three risk factors of fluoride bone injury and two metabolism indicators of bone metabolic disorders were included. The major predictor in the model was calcitonin receptor gene polymorphism (OR=8.725). It suggested that occupational physicians could detect the calcitonin receptor gene polymorphism in pre-employment health examination to avoid people with TC&TT genotype from engaging in the work exposed fluoride. Early fluoride bone injuries had no X-ray changes and were difficult to be diagnosed in occupational physical examinations. We believe that if the model applied to workers exposed to fluoride (not diagnosed as fluoride bone injuries in occupational physical examinations), those who at high-risk or early stage for fluoride bone injury would be identified. According to this model, we can set thresholds for intervention. For example, we set predicted probability of developing fluoride bone injury 0.649 as a threshold. Workers got 0.649 or higher predicted probabilities would be suggested to change their work category (to the work not exposed to fluoride) to prevent fluoride bone injury.

Our study had limitations. The statistics used to develop the risk prediction model were complicated and some of the variables used in the model are not readily available, precluding the ability of an occupational physicians to determine a worker's fluoride bone injury risk. Another potential limitation of this study is that the data used to develop the model were from 2011. There have not been any notable changes in fluoride bone injury risk factors since 2011, indicating the model should still be valid today. Once risk factors change, that will impact the predictive capability of the model. This model however could be considered as a starting point for further development.

In summary, we have derived and validated a risk prediction model which estimates the likelihood of undiagnosed fluoride bone injury in workers exposed to fluoride. The prediction model has the potential to be a useful tool in the prevention of fluoride bone injury. Calcitonin receptor gene polymorphism was the major predictor. The next step for this project is longitudinal validation of the model's predictive ability among workers exposed to fluoride. If the predictive accuracy of the model proves as good in a longitudinal dataset, the model could be used to assess the fluoride bone injury risk, and identify the susceptible workers. Then more occupational interventions could be designed to protect susceptible workers from fluoride bone injury.

No potential conflict of interest relevant to this article was reported by the authors

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