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Sub-micrometer Scale Synchrotron X-ray Fluorescence Measurements of Trace Elements in Teeth Compared with Laser Ablation Inductively Coupled Plasma Mass Spectrometry

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Abstract

Background: Elemental analysis of teeth allows for exposure assessment during critical windows of development and is increasingly used to link early life exposures and health. The measurement of inorganic elements in teeth is challenging; laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is the most widely used technique.

Objective: Both synchrotron x-ray fluorescence (SXRF) and LA-ICP-MS have the capability to measure elemental distributions in teeth with each having distinct advantages and disadvantages.

Methods: In our study, we compared these two methods for teeth elemental quantification. SXRF was able to achieve spatial resolutions of 0.3 μm and is non-destructive while giving similar elemental quantification results to LA-ICP-MS.

Results: For particular elements, SXRF can offer lower detection limits but depends on the specific beam intensity. The comparison between methods revealed less than 10% disagreement between quantification results from LA-ICP-MS and SXRF.

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Author Contributions

AJ Specht: Conceptualization, drafting, reviewing; X Zhang: Drafting, reviewing; OA Antipova: Drafting, reviewing; ASM Sayam: Drafting, reviewing; VY Nguyen: Drafting, reviewing; CG Hoover: Drafting, analysis, reviewing; T Punshon: Drafting, reviewing, conceptualization; BP Jackson: Drafting, conceptualization, reviewing; MH Weisskopf: Drafting, conceptualization, reviewing.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics Approval and Consent to Participate

The study received IRB approval from Harvard University and adhered to all guidelines and regulations set forth. Informed consent was obtained from all study participants.

Significance: Overall, either measurement modality could be used to effectively measure elemental distribution in teeth to aid in epidemiological studies of early life exposures.

1. Introduction

Inorganic elements have a variety of functions in living organisms. Essential elements such as zinc (Zn) and iron (Fe) play critical roles in biological processes and an optimal level is needed for proper function. Non-essential, potentially toxic elements, such as lead (Pb) and arsenic (As), have no biological function, and exposures to these cause adverse health effects.(1,2) The acute and chronic health implications of potentially toxic elemental exposures underlie the need to collect biomarkers of exposure.(1) Typically, biomarkers for exposure to inorganic elements reflect only recent exposures, such as blood (hours-days), urine (several days), hair (months), or toenails (months), with blood being the most common biomarker used in exposure studies (3–5). The half-life of many elements in these tissues is relatively short, on the order of days to months (6–9). There are fewer biomarkers of cumulative exposure to inorganic elements over much longer periods which would be better biomarkers in epidemiological studies of chronic health outcomes. Pb in bone and cadmium (Cd) in urine are two such examples. Biomarkers of early life exposures not influenced by recent exposures are more challenging to collect. These types of biomarkers are particularly valuable for studies of the developmental origins of health and disease in adults, an area of growing interest (3,10,11).

Teeth, which accumulate inorganic elements during active growth and development, have been used in health studies as a means to capture exposures ranging from fetal to adolescent life stages (10,12). Early studies of teeth used ground, whole teeth to measure elemental or radioisotope accumulation (11,13,14). For this study, we are particularly interested in tooth enamel which is primarily composed of hydroxyapatite crystal mineral.(15) The mineralization process in tooth enamel occurs between 3 months to 16 years of age depending on the individual tooth development.(16,17) Upon completion of enamel, the elements and isotope ratio inside the enamel matrix remain unchanged throughout the lifetime.(10) It makes tooth enamel an appropriate biomarker to assess the diet, elemental exposure, and even geographical origin of the subject during the developmental window unlike other biomarker like bone which typically undergoes several reformations during adulthood.(18) (3,10,12). Thus, information on the timing of exposure could potentially reveal how exposures to inorganic elements during critical developmental windows affect later life health outcomes (3,10,12).

Recent studies have used laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) of deciduous teeth (3,10,12,19,20). LA-ICP-MS has been used on teeth sections to obtain a record of exposure relative to the day of birth (21,22). However, the spatial resolution of this technique is currently limited by the laser to 4 µm resolution at the lowest, with most operating at 10–20 µm (21). Fine-resolution assessment using synchrotron x-ray fluorescence (SXRF) could be used to assess exposure levels down to a daily basis or below. The resolution of SXRF, although dependent on the beamline, can be on the order of 0.3 µm (23). SXRF may provide the opportunity to disentangle complex exposures with higher resolution, which will aid studies of cyclic metabolic processes, saving time by mapping

faster than typical LA-ICP-MS (19,20). SXRF methods have been used effectively for elemental mapping of tissue and teeth in other studies (24–26).

In addition, SXRF has the advantage of using a calibration that does not require matrix-matched composition for the quantification of elements, whereas LA-ICP-MS typically requires matrix-matched controls to verify quantitative units. Around the world, SXRF systems vary considerably in terms of spatial resolution and limit of detection capabilities. In this study, we used the 2-ID-E Microprobe at Advanced Photon Source at Argonne National Lab, which has a sub-micron spatial resolution. High-resolution SXRF is done at large facilities, typically at national labs in the US, that now use micrometer spatial resolution with high enough intensity to measure elemental exposures in teeth (24–26). Importantly, LA-ICP-MS has the advantage of accessibility through in-lab measurements, whereas SXRF typically requires beamline access through a typically long application and review process which can hinder accessibility greatly. LA-ICP-MS, as a lab-based method, is much easier for novel sample measurements with an ever-increasing number of labs acquiring the technology. Finally, detection limits for each methodology can vary. LA-ICP-MS for a single pixel of data will be able to detect ppm concentrations. SXRF will vary based on the synchrotron used to collect data, the detectors used, and the spatial resolution, but the samples measured here would have 0.1 ppm level detection limits.

In this study, we compared LA-ICP-MS and SXRF for the measurement of inorganic elements in teeth, which were imaged to identify pre- and post-natal exposure periods in the enamel. We demonstrate the similarities of overall elemental quantification within the measured areas of the tooth and discuss the advantages and disadvantages of each technique.

2. Methods

2.1 Tooth Samples

We examined three lower incisor teeth collected in the 1950s from male participants (14). These teeth were originally collected as part of the St. Louis baby teeth study.(14) The study involved collections of teeth from all across the US with particular emphasis in the St. Louis area of Missouri in the US. They were stored in envelopes from that time until now. Teeth were embedded in a cold-setting epoxy resin (Electron Microscopy Sciences Epofix™ Embedding Resin) and sectioned with a diamond wafering blade along the para-sagittal plane. The block was polished (SiC paper grades 12 µm, 9 µm, and 1 µm for 1 minute each) and sonicated in DI water to remove debris. Blocks were glued to a petrographic slide (Devcon 5-minute Epoxy) and sectioned again to leave a 250–400 µm section on the slide. And sections were polished using the same paper series, sonicated in DI water, and allowed to dry overnight. The sections were 0.23, 0.61, and 0.52 mm thick respectively. Microscope imaging was used to identify the neonatal line and pre-and post-natal areas within the enamel. The teeth were further imaged using macro-lens and scale to identify lines for measurement with LA-ICP-MS and anatomical features for identification with SXRF.

2.2 Synchrotron X-ray Fluorescence

The SXRF measurements were conducted at the Argonne National Laboratory Advanced Photon Source beamline 2-ID-E. This is a synchrotron beam source, where particles accelerate in a circular pattern and along the edges, different beamlines can do research related to measurements made by the strong x-ray radiation produced while the particles move through the circular path. This beamline reaches spatial resolutions of $0.5 \times 0.3 \mu\text{m}$ using a Fresnel zone plate to focus the X-ray beam. We used an incident energy of 15.8 keV (15–16 keV) to detect lead L emission lines. The following elements would give signal and technically be quantifiable from the sample as well: F, Na, Mg, Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Mo, Rh, Ag, Cd, Pb, Hg, Au, W, Ba, U, and Th. Take note, this is dependent on biological accumulation being above the detection limits to produce data distinguishable from background data. The glass slides were cut and attached to a kinematic sample mount using clear nail polish, which was then placed into the sample positioning apparatus for movement in the beam and fine scanning during XRF mapping measurements. Tooth samples were first scanned using $30 \times 30 \mu\text{m}$ resolution with a 5 msec time per pixel to identify the areas of the tooth. Then a fine scan with $3 \times 3 \mu\text{m}$ resolution and 50 msec time per pixel was used to get the most accurate data from a section of the enamel.

The metal quantification was obtained from calibration to an Applied X-ray Optics thin film SXRF reference standard (RF8-200-S2455) with known elemental compositions. The spectral results were normalized to the downstream and upstream ion chamber counts to account for differences in thickness across the tooth section. Per-pixel spectral fitting was completed using MAPS, which is a custom-built software package produced in IDL to process spectral data per pixel from the synchrotron XRF output and produce elemental maps (22). The output units of the spectral analysis for elemental quantification were $\mu\text{g}/\text{cm}^2$. Line data was obtained from SXRF by identifying the equivalent section of the enamel to the LA-ICP-MS data in the total measured tooth. To do this, we matched the lines from Figure 1 onto the elemental maps generated from SXRF in Figure 2, then we could output scatter data (point by point across the line) and average statistics for each element across the line. This point-by-point and average line data was used in the comparison to LA-ICP-MS for the final analysis. We also used a comparison between line data and the total pre- or post-natal area identified via a microscope image of the neonatal line. We similarly did a comparison between the imaged neonatal line and the elemental map produced by SXRF to identify the total pre- and post-natal areas for this analysis.

2.3 Laser Ablation Inductively Coupled Plasma Mass Spectrometry

The LA-ICP-MS was performed at Dartmouth College using an Agilent 8900 triple quadrupole ICP-MS and an NWR213 laser ablation system (ESI, Bozeman, Montana). This system uses a standard ICP-MS system coupled with a laser. The laser ablates a small amount of material off a sample (approximately 30 ug), which can then be input to the ICP-MS directly. ICP-MS then measures elements using a mass to charge ratio, which will differ each element's movement through quadrupoles in the system. The elements measured from this technique are Be, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Tl, Hg, Pb, and U. Take note, this is dependent on biological accumulation

being above the detection limits to produce data distinguishable from background data. Data was collected along predetermined lines of pre-and post-natal areas at a spatial resolution of 25 μm (Figure 1). The laser was operated at 100% power, 100 μm per second, and 20 Hz. Two passes were used to move potential external contamination. Standard data from NIST1486a was collected for quality assurance and to correct measures to parts per million from instrument drift. NIST 1486a is a bone meal standard reference material from the National Institutes of Standards and Technology in the US. Results are ratioed to calcium and then compared with NIST1486a to calculate final measures in parts per million.

2.4 Statistical Analysis

The statistical analysis was performed using R statistical software, version 3.0.3 (Foundation for Statistical Computing, Vienna, Austria). We first used ordinary least squares linear regressions to determine whether the elemental quantification between LA-ICP-MS (independent variable in the regression) and SXRF (dependent variable in the regression) was equivalent. The beta values indicate whether both methods equivalently measure each of the elements. We calculated Pearson correlations on an elemental basis over line measurements and total enamel areas, comparing the average values of each element measured by LA-ICP-MS and SXRF. The beta coefficient from regressions should give us a comparison between instrument quantification methods. A beta coefficient closer to one would indicate near perfect agreement between quantification from either method, whereas a beta coefficient further from one would indicate they come to different conclusions in terms of quantification. Both quantification methods should be giving units comparable by a 1:1 ratio. P-values from this relationship would indicate the statistical likelihood of the instruments providing different quantitative results from the same sample. Thus, a significant p-value would demonstrate that both systems have a degree of certainty in the quantification to make them statistically equivalent.

Finally, we compared between the total pre- or post-natal area and the line measurements. We used Wilcoxon rank sum tests to determine differences between LA-ICP-MS lines and XRF total pre- and post-natal areas. This gave us an appropriate comparison between two methods for measuring elemental abundance teeth and identification of potential differences between using a single line that should average exposure over pre- or post-natal developmental time windows respectively, versus the total area reflective of that period that is easily captured by SXRF. Similarly, we compared the LA-ICP-MS line data and SXRF total area to determine quantitative differences, and correlations to see if there are any variations within the full measurement area.

3. Results

3.1 SXRF Measures of Teeth

3.2 Average Elemental Concentrations

Averages for the SXRF data and LA-ICP-MS data of the three teeth are displayed in Table 1 below. LA-ICP-MS data is in parts per million calibrated to a NIST 1486 standard. SXRF is calibrated to gram of element per area.

3.3 Comparison of total elemental quantification between LA-ICP-MS and SXRF

The correlations between data for each element test the overall agreement between LA-ICP-MS and SXRF data. These correlations used the mean part per million values of P, Fe, Ni, Cu, Zn, and Pb from LA-ICP-MS over a line measurement and compared them to the mean values of those elements obtained using SXRF in $\mu\text{g}/\text{cm}^2$. Table 2 shows the overall agreement between the LA-ICP-MS and SXRF data. Perfect agreement between the approaches would result in a beta estimate of one.

3.4 Comparisons of Detection Limits and Spatial Resolution

The detection limit SXRF measurements of Pb at a dwell time of 50 milliseconds was ~ 0.6 ppm per pixel. The spatial resolution for SXRF for these measurements was $3 \mu\text{m}$, with the lowest option of $0.2 \mu\text{m}$ at the time of our measurements on beamline 2IDE. LA-ICP-MS was done using $25 \mu\text{m}$ with capabilities down to $4 \mu\text{m}$. Additionally, the SXRF gives us the option for increased scan times to decrease detection limits if necessary.

4. Discussion

This study sought to compare two elemental imaging methods for the measurement of teeth as retrospective biomarkers of inorganic element exposure and demonstrated the feasibility of using SXRF to evaluate elemental distributions in teeth. SXRF was compared to standard LA-ICP-MS. We found the SXRF is comparable to LA-ICP-MS for quantification of elements and demonstrated agreement between SXRF in $\mu\text{g}/\text{cm}^2$ and calcium-normalized LA-ICP-MS data over pre- and post-natal areas. Quantification between LA-ICP-MS and SXRF was comparable, with both methods displaying the ability to measure the concentrations of a suite of elements in the tooth.

SXRF data over the enamel areas in the teeth had close relationships with the quantification from LA-ICP-MS data. This was despite the key differences in the sampling methodology. SXRF should measure slightly deeper into the tooth, whereas LA-ICP-MS will take only a small amount of material from the surface for measurement –estimated as $1 \mu\text{m}$ depth per second at 75% power. The escape depth for even the highest energy characteristic x-rays (Pb) of our elements of interest would represent an average depth of 0.2 mm ($200 \mu\text{m}$), as taken from NIST attenuation coefficients (27). For other elements in the XRF spectra with characteristic energies of 5 keV or less, the average penetration depth would be $20 \mu\text{m}$ according to NIST attenuation coefficients (27). This would produce a bias error in comparison to the LA-ICP-MS data, which is all measured at the same depth. Ideally, this difference in depth for SXRF should not cause issues in quantification since calibration accounts for these changes based on energy and penetration depth. Nonetheless, we found no differences in our quantification based on sample thickness.

The elements we chose for comparison represent those that were easily measured by both methods. Certain elements of interest, like arsenic or mercury for example, were not accurately quantified on LA-ICPMS, whereas an element like strontium was not included in our SXRF analysis because the beamline energy was set at 15.8 keV which would not have been enough energy to excite the strontium K-line with an initial binding energy of 16.1

keV. Future work should focus on more samples with direct comparisons of more variability of elements that were unable to be detected in this work, and, thus, not compared between the methods.

This study did have some limitations, which should be addressed in future work. First, the sample size was very small with only three total sample measures. Despite this, measurements on a micrometer scale provided plenty of data points for comparisons and validation of methodology, and this is important for the use of both techniques in future studies. The findings shown here support the use of both SXRF and LA-ICP-MS in future exposure assessment studies of teeth and health outcomes. SXRF and LA-ICPMS both have different advantages. Specifically, SXRF teeth exposure assessment has the advantages of being non-destructive and having finer spatial resolution, but the disadvantage of being more challenging to access, and elemental accuracy dependent on beam properties. LA-ICP-MS has the major advantage of being a benchtop instrument but can have less sensitivity for certain elements (anything lighter than Be).

Overall, SXRF and LA-ICP-MS are both suitable for 2D spatial exposure assessment in teeth. SXRF offers some significant advantages over LA-ICP-MS in non-destructive measures, and lower spatial resolutions, but has the significant disadvantage of needing to be at a synchrotron source for measurement. Although the lab-based 2D x-ray fluorescence approach is on the rise, it may open up the technology to become more widely available in lab settings. In the future, more studies utilizing SXRF would benefit the epidemiological evidence of finer temporal exposures and their impacts on health or metabolic cycles.

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Data Availability

Data will be made available upon reasonable request.

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Impact Statement:

Synchrotron x-ray fluorescence can be used to effectively quantify elemental distributions in teeth at a nanoscale resolution and is comparable to current laser ablation inductively coupled plasma mass spectrometry. Both methods offer advantages and disadvantages with LA-ICP-MS offering in-lab analyses, whereas SXRF offers much finer spatial and temporal scales and better detection capabilities. For studies focused on fine scale changes in structure, SXRF is more appropriate than LA-ICP-MS.

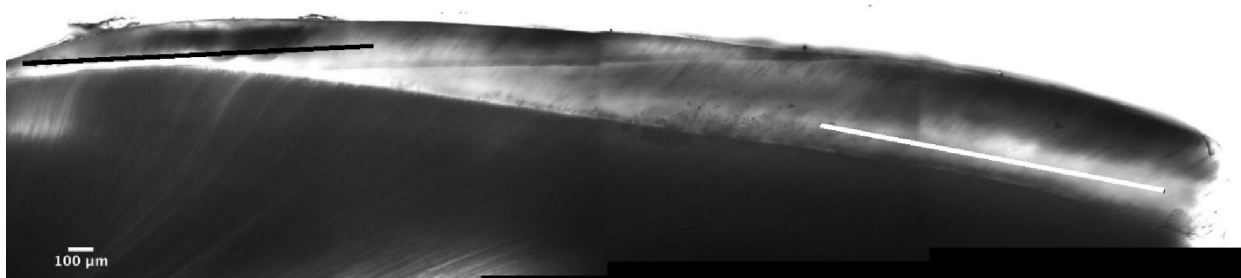
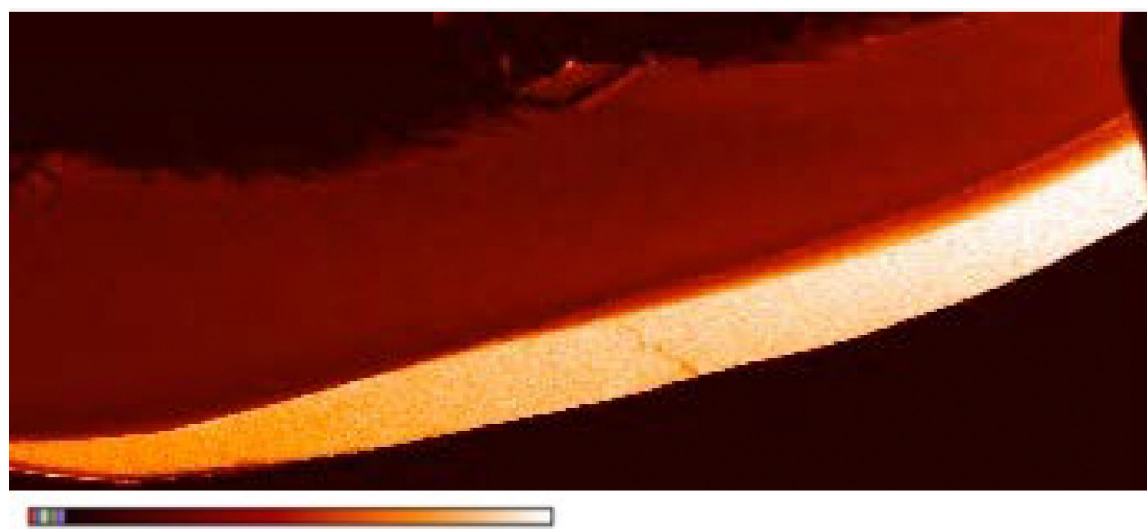
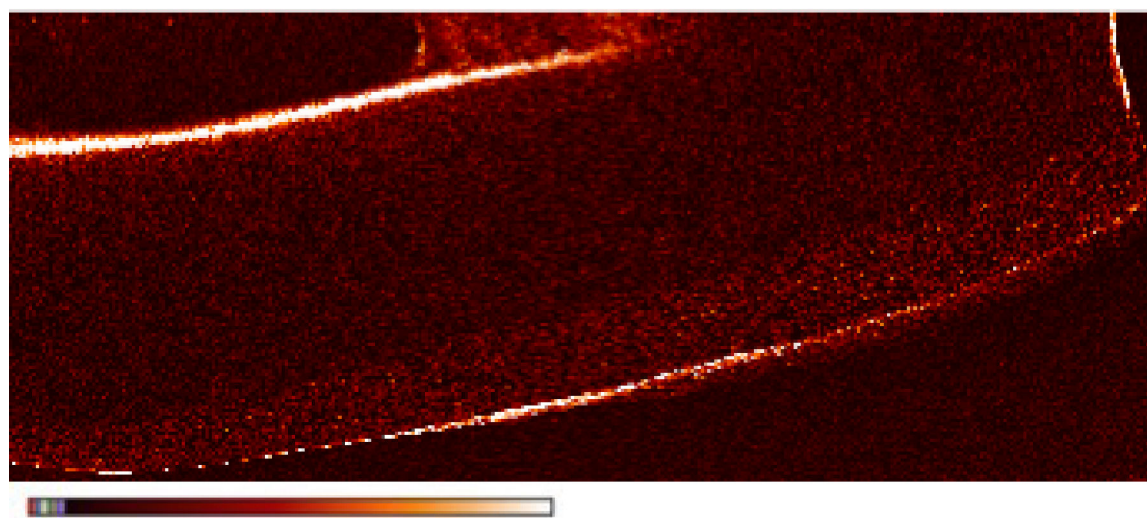


Figure 1.
Example of line data from LA-ICP-MS taken from tooth enamel representative of pre- (black line) and post-natal (white line) areas on the tooth with the black arrow indicating the neonatal line hitting the enamel surface.



A



B

Figure 2.

A) Calcium distribution (Darker at 0 to brighter at $10253 \mu\text{g}/\text{cm}^2$) in enamel obtained using SXRF measurements **B)** Lead distribution (Darker at 0 to brighter at $123 \mu\text{g}/\text{cm}^2$) in enamel obtained using the SXRF measurements in the same tooth as above.

Table 1.

Average values were obtained for elemental concentrations via LA-ICP-MS and SXRF.

	LA-ICP-MS (ppm)		SXRF ($\mu\text{g}/\text{cm}^2$)	
	Average	Standard Deviation	Average	Standard Deviation
<i>P</i>	628453.00	15536.55	1618.8	789.3
<i>Fe</i>	3694.75	218.63	1.6	0.5
<i>Ni</i>	2.86	0.13	2.4	0.8
<i>Cu</i>	794.13	12.82	1.0	0.3
<i>Zn</i>	14002.63	480.39	5.5	6.1
<i>Pb</i>	44.38	2.27	2.2	2.0

Table 2.

Correlation between elemental concentrations in teeth using LA-ICP-MS and SXRF measures of the total pre and postnatal areas.

Tooth Number and Enamel Location	Total Pre- or Post-Natal Area		
	Beta	R ² Value	P-Value
<i>Sample 1 Pre-natal</i>	0.92	0.92	<0.001
<i>Post-natal</i>	0.91	0.91	<0.001
<i>Sample 2 Pre-natal</i>	0.93	0.93	<0.001
<i>Post-natal</i>	0.92	0.92	<0.001
<i>Sample 3 Pre-natal</i>	0.93	0.94	<0.001
<i>Post-natal</i>	0.94	0.94	<0.001