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Transition and post-transition metals in exhaled breath condensate

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Abstract

Water vapor in expired air, as well as dispersed non-volatile components, condense onto a cooler surface after exiting the respiratory tract. This exhaled breath condensate (EBC) provides a dilute sampling of the epithelial lining fluid. Accordingly, the collection of EBC imparts a capacity to provide biomarkers of injury preceding clinical disease. Concentrations of transition and post-transition metals in EBC are included among these endpoints. Iron and zinc are those metals in highest concentration and are measurable in all EBC samples from healthy subjects; other metals are most frequently either at or below the level of detection in this group. Gender, age, and smoking can impact EBC metals concentrations in healthy subjects. EBC metals concentrations among patients diagnosed to have particular lung diseases (e.g. asthma, chronic obstructive pulmonary disease, and interstitial lung disease) has been of research interest but no definite pattern of involvement has been delineated. Studies of occupationally-exposed workers confirm significant exposures to specific metals but such EBC metals measurements frequently provide evidence redundant with environmental sampling. Measurements of metals concentrations in EBC remains a research tool into metal homeostasis in the respiratory tract and participation of metals in disease pathogenesis. Quantification of metals concentrations in EBC is currently not reliable for clinical use in either supporting or determining any diagnosis. Issues that must be addressed prior to use of EBC metals measurements include the establishment of both standardized collection and measurement techniques.

Keywords

Lung; transition elements; metals; iron; zinc

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Introduction

As a result of a large surface area (50 to 75 m^2), a considerable volume of water is lost daily from the human body through its evaporation from the epithelial lining fluid of the respiratory tract (1). While much of this volume is water vapor, a small fraction represents aerosol droplets generated through an action of turbulent flow on the epithelial lining fluid (2) or by opening and closing of small airways (3). Following this aerosolization, non-volatile molecules are dispersed into the water vapor. Water vapor in expired air, as well as the dispersed non-volatile components, condense onto a cooler surface after exiting the respiratory tract. Instrumentation can be added to lower the temperature of the surface and augment collection. This liquid sampling obtained from a subject, typically breathing at tidal breathing, is exhaled breath condensate (EBC).

The human respiratory tract represents the route of entry for many environmental and occupational exposures. Accordingly, volatile and nonvolatile substances in EBC can function as biomarkers as concentrations can be impacted by these exposures. Such biomarkers may potentially precede evidence of injury and therefore predict clinical disease. Concentrations of transition and post-transition metals in EBC are included among these endpoints.

Metals in the respiratory tract

Transition and post-transition metals were selected in molecular evolution to carry out a wide range of biological functions and are required by all living organisms. They are utilized in almost every aspect of normal cell function and are particularly crucial for cellular metabolism. Consequently, almost all living organisms require metals including iron, zinc, copper, chromium, cobalt, manganese, molybdenum, nickel, tin, and vanadium.

Cells resident in the lung express proteins which participate in the import, export, storage, and transport of metals (e.g. divalent metal transporter I, ferroportin I, ferritin, and transferrin receptor respectively) (4). Metal uptake by non-diseased, non-exposed lung with systemic translocation has been demonstrated (5). However, the positioning of these proteins does not support metals uptake with systemic translocation to meet nutritional requirements as a normal function for which the lung was designed (6).

While all cells in the lower respiratory tract participate in metals homeostasis, handling of metals is a primary responsibility of airway and alveolar macrophages (7, 8). The macrophage has a capacity to mobilize, import, store, and release metals for transport to tissues of the reticuloendothelial system (e.g. liver). The overwhelming majority of metals in the lung can be localized to macrophages (9). Stains reflect this localization of metals in the respiratory tract to macrophages (10). The macrophage, with its accumulated metals, can be removed from the lung via mucociliary clearance and lymphatics.

Metals homeostasis in the lung is a dynamic rather than a static process. Following exposure of the respiratory tract, metals are removed and some portion can be translocated outside of the lungs reaching other tissues of the body (11). This movement of metals from the respiratory tract into the systemic circulation with distribution to numerous different tissues is observed with particle-associated metals (12). Such translocation from the respiratory tract can occur rapidly (e.g. over hours). Those metals with higher water solubility are more quickly transported systemically relative to those in an insoluble state. Clearance from the respiratory tract may also correlate with the availability in the lung of reductants and chelators which function to solubilize metals. Measurements of metals in samples of the lung will reflect the efficiency of clearance of the metals and their systemic translocation. Accordingly, metals concentrations in the respiratory tract will be dependent on duration of time since the initial exposure.

Collection of exhaled breath condensate

The American Thoracic Society and European Respiratory Society (ERS) have provided guidelines for the collection of EBC (13). Such collection necessitates a subject breath through a one-way valve into a tube cooled to condense exhaled water vapor. A collecting period of 10 minutes or more provides a volume of condensate suitable for metals analysis (2 to 3 mL). The device can be integrated with a pneumotachograph allowing a definition for the completion of collection as a total expired volume rather than a duration of time (14). A device with audio and visual prompts can also be employed to control respiratory rate, tidal volume, and expired volume and to terminate the effort (15).

At the low concentrations measured in EBC, metals contamination during the collection is a concern. The specific collection device can impact metals concentrations (16, 17). To minimize metals contamination, the condensing surface should be a plastic polymer or fluoropolymer. The plastic ware which the EBC is collected into should be disposable. Alternatively, components can be acid washed prior to use in an effort to diminish metals contamination.

Contamination of EBC due to metals included in particles in ambient air should be considered and eliminated if possible. All metals in the atmosphere, except for mercury, are associated with particles (18). If one assumes 1) a tidal volume of 0.5 L, 2) a respiratory rate of 20/minute, 3) a collection time of 10 minutes, 4) an ambient particulate level of 20 μg/m^3, 5) a metals composition in the particles of 1%, and 6) lung deposition of particles which approximates 50%, it is calculated that with collection of the exhaled particles, the metals concentrations could approximate levels of parts per billion (19):

1) 0.5 L/breath (tidal volume) × 20 breaths per minute × 10 minutes = 100 L or 0.1 m^3 (inspired volume over 10 minutes of collection)

2) 0.1 m^3 (inspired volume) × 20 μg/m^3 (particle level) = 2 μg (particle mass in the inspired volume over 10 minutes of collection)

3) 2 μg (particle mass) × 50% (percentage not retained and expired)/1 mL (volume of EBC) = 1 μg particle/2 mL EBC

4) 1 μg particle/2 mL EBC × 1% metals = 5 ppb metals (mass per volume)

With investigation providing measurements of metals in parts per trillion, particles in the ambient air potentially impact results. This is particularly true with occupational studies where the ambient particle levels are elevated and there is higher metals content (e.g.

welders). An appropriately-sized filter attached to the inlet of the breathing train can be used to reduce exposure to particles during EBC collection.

Measurements of metals concentrations in exhaled breath condensate

The methodologies most frequently employed to measure transition metals in EBC currently include graphite furnace-atomic absorption spectrometry (GF-AAS) and inductively coupled plasma mass spectroscopy (ICPMS). While there are advantages to each, the costs of the equipment are high and the choice of which to use in metals measurements in EBC is frequently dictated by availability. Ultrapure water must be obtained and used for dilution of stock solutions and to prepare blanks. Multi-element solutions can be purchased for external standards. An internal standard (e.g. yttrium) is recommended. Results can be reported as metals per volume condensate (i.e. microgram per mL and parts per billion (ppb)) rather than metals per expired volume as the latter is difficult to interpret and will reflect variability of both the metals measurements and the spirometer. Both instrument detection limits and method detection limits should be calculated.

Metals in EBC should not be measured prior to acidification/digestion; the acid employed (HCl or HNO_3) should be very low in metals (e.g. Optima grade, Fisher Scientific). A direct assay of EBC for metals without prior homogenization/digestion will not provide an accurate analysis (20).

A significant increase in metals concentrations in samples was reported after their storage for several weeks (17). This was presumed to be the result of leaching from the storage containers. Accordingly, it is currently recommended that the samples be analyzed as soon as possible.

Metals analysis of EBC can result in levels which are below the method detection limit in a majority of the samples (17). Investigations have reported aluminum, cadmium, chromium, and tungsten levels to be below the limits of detection in a majority of samples (16, 21–24). Subsequently, group effects can frequently indicate the number of samples with detectable concentrations (24). Such findings must be interpreted with some caution.

Metals in EBC in normal populations

The epithelial lining fluid of the respiratory tract (i.e. the alveolar and airway lining fluids) is most commonly sampled using bronchoalveolar lavage (BAL) and EBC. Regarding dilutions, it is estimated that approximately 1.0 +/− 0.1 mL of epithelial lining fluid was recovered per 100 mL BAL fluid (25). In view of this, BAL fluid is considered an approximately 1/100 dilution of the epithelial lining fluid. The EBC is a more dilute sampling of the epithelial lining fluid (26). Based on 1) the dilution of the BAL fluid approximating 1:100 and 2) the total protein concentration in BAL fluid and EBC approximating 100 and 1 μg/mL respectively, an estimate of the dilution of epithelial lining fluid that EBC exhibits would be 1: 10,000 (27, 28). Similar dilution estimates have been calculated using urea or electrolyte (sodium and postassium) as dilution markers (29, 30). This dilution of EBC appears to be extremely variable both among individuals and within an individual over time (29–31). This may correspond to changes in ventilation and condensation temperature as the main determinants of evaporation and efficiency of collection respectively (32), or to individual differences in abilty to generate aerosols of airway lining fluid (33).

Metals which have been quantified in BAL fluid collected from healthy subjects are few (Table 1) (34–43). Iron, zinc, copper, and chromium have been those metals measured most frequently in BAL fluid from healthy subjects. The range of metals concentrations in these individuals is wide indicating the different methodologies employed in performing both the procedure and the analysis. In addition, dissimilar populations of healthy subjects were used and could include nonsmokers, smokers, and even patients. Despite the widely disparate approaches, it is accepted that iron and zinc are those metals in greatest concentrations in BAL fluid with copper being less. It is uncertain that other metals are at quantifiable levels in BAL fluid collected from healthy subjects. This does not negate a significant role for other metals in the human lung of both healthy and diseased individuals but defining metal participation in a biological effect may be problematic. While metals accumulate with age in numerous tissues including the lung, increases in BAL fluid metals among older, healthy subjects has not been shown (44, 45). Smoking does increase levels of some metals in BAL fluids (40, 42). Comparison of metals concentrations in BAL fluid with those in blood supports the possibility of blood being a source for iron, zinc, and copper in the epithelial lining fluid (46–49). However, all other metals in BAL fluid are reported at approximately comparable levels as in blood and it is proposed that environmental sources (e.g. air pollution particles, environmental tobacco smoke, and smoking) may impact these concentrations greater than those in the vascular compartment.

EBC has numerous advantages over BAL fluid as a sampling of epithelial lining fluid in that its collection 1) does not demand a difficult preparation, 2) is non-invasive, 3) requires a short duration of time, 4) can be repeated multiple times, and 5) can be based outside of medical clinic or hospital (26, 50). Accordingly, metals have also been measured in EBC collected from healthy subjects (Table 2) (14, 17, 51–57). Comparable to BAL fluid, iron and zinc are those metals in highest concentration and are measurable in all EBC samples. However, other metals in EBC samples are much lower in concentration and a majority of the samples can be below the level of detection (17, 54). Gender, age, and smoking can impact EBC metals concentrations (16, 17, 22, 24, 52, 58). Differences between genders and with aging can reflect the volume of collected condensate which is proportional to the ventilatory volume (pulmonary function in healthy non-smokers is determined by gender, age, and height/weight). Tobacco smoke includes metals and smoking can be predicted to introduce some quantity of metals into the respiratory tract (59). Lung tissue from smokers demonstrated elevated concentrations of iron (45). Current healthy smokers can have higher EBC concentrations of lead and cadmium relative to healthy nonsmokers (50). In smokers, iron concentrations in EBC were significantly increased when compared to healthy controls (60). Cadmium, lead, and aluminum levels in EBC were higher among smokers and smokers with COPD (61). When patients with chronic obstructive pulmonary disease (COPD) were subdivided into smokers vs. ex-smokers and nonsmokers, the smokers were shown to have higher EBC lead, cadmium, and aluminum levels (52). Ex-smokers with COPD who had quit smoking for more than 2 years continued to demonstrate elevations in EBC metals relative to non-smokers (50). Interestingly, reduced concentrations of iron and nickel can also be observed in the EBC of smokers (16, 52). Finally, some investigation demonstrated that exposure to cigarette smoke does not always impact metals concentrations in EBC (16).

Metals in EBC in diseased populations

Metals in EBC samples have been measured in patients diagnosed with asthma, chronic obstructive obstructive disease (COPD), and interstitial lung disease. Among 50 healthy subjects and 30 asthmatics, the measurement of metal concentrations supported lower iron levels in the group of asthmatics (52). There were no differences in concentrations of lead, aluminum, cadmium, copper, and manganese. Comparable results were observed in EBC collected from 22 healthy children and 17 asthmatics with the latter having a statistically significantly lower iron concentration (55). Another study with a small number of participants observed no significant differences in EBC iron concentration between asthmatics (n= 10) and non-asthmatics (n=16) (62). Using a “bleomycin technique for measurement of pro-oxidant iron”, there was an increase in EBC iron levels post-exposure to city environments among severe asthmatics (60). These studies do not clearly define a participation of metals in the pathogenesis of asthma but do suggest that further investigation into a role for iron in the induction of asthma is warranted.

EBC obtained from patients with stable COPD patients (n= 50) revealed higher concentrations of lead, cadmium, and aluminum, and lower levels of iron and copper relative to samples collected from healthy subjects (n= 50) and healthy smokers (n= 30) (52). No correlations were observed between indices of pulmonary function and EBC metals concentrations. When the COPD patients were classified on the basis of disease severity, associations with differences in EBC metals were not demonstrated. Decreased EBC iron levels in COPD patients, relative to smokers with no obstruction, were attributed to a failure of the diseased lung to excrete iron (63). EBC collected in 28 COPD patients presenting in mild to moderate exacerbation revealed increased manganese concentrations relative to samples at recovery (64). Based on this investigation, it is uncertain if metals participate in the etiology or contribute to exacerbation of COPD. Further studies of an association between metals and COPD are needed, particularly with a focus on iron and copper.

Levels of metals were quantified in EBC from patients with interstitial lung diseases (54). Among patients with sarcoid (n= 22), non-specific interstitial pneumonia (n= 15), and idiopathic pulmonary fibrosis (n= 19), concentrations of chromium and nickel could be increased relative to levels observed among healthy subjects (n= 33). Elevated EBC concentrations of chromium and nickel among the interstitial lung patients disagreed with observations in COPD patients implying that the two groups were different despite smoking being a major risk factor for both. In contrast, both EBC iron and copper were decreased in groups with interstitial lung disease. The observed decrements in metals concentrations in the EBC could reflect a reaction comparable to the hypoferremic response observed in the serum with inflammation.

Metals in EBC in occupational and environmental studies

The analysis of EBC endpoints has been described as one of the most promising methods available for the study of pulmonary biomarkers of exposure, effect, and susceptibility in occupational settings (58). Metals in EBC have been measured as biomarkers to evaluate occupational exposures including those among welders and workers in the hard metal, chrome plating, lead processing, and aluminum production industries.

Samples of EBC from 45 welders showed elevated levels of aluminum, nickel, and chromium relative to 24 non-exposed control subjects (65). EBC from welders revealed high iron and nickel concentrations (22). Concentrations of manganese and nickel in EBC were significantly higher among 17 welders compared to 16 unexposed control subjects after 5 days’ exposure (14). Welders showed significantly higher concentrations of iron and nickel in EBC relative to non-exposed volunteers (66). Dissimilar working conditions between different companies impacted elevations in iron and nickel concentrations in the EBC collected from 36 welders (16).

A second occupation with metals exposure which has been investigated employing EBC endpoints is hard metal industries. Thirty-three workers in workshops producing either diamond tools or hard-metal mechanical parts showed detectable cobalt levels in the EBC while tungsten was undetectable (21). In contrast, EBC concentrations of cobalt and tungsten were reported to be measurable but not significantly elevated among 62 workers at a hard metal processing plant (67).

Metals concentrations in EBC have been utilized as endpoints in studies of several other industries including chrome plating, lead processing, and aluminum production. EBC from groups of 10 and 24 chrome platers supported measurable chromium levels (51, 68). Chromium levels measured in EBC correlated with those measured in red blood cells among 14 non-smoking, male chrome-platers (69). EBC samples collected from a cohort of 58 workers occupationally exposed to chromium compounds and 22 unexposed volunteers showed significantly higher levels of chromium in the former (57). In a group of workers from two lead processing plants, lead concentrations in the EBC reflected the levels of the metal in the work environment settings (70). Among workers in an aluminum production plant, EBC concentrations of beryllium and aluminum were higher in pot room workers when compared with controls (23).

Finally, metals concentrations have been quantified in EBC collected from workers 1) exposed to titanium dioxide (TiO_2) and 2) at an airport. EBC collected from 20 workers exposed to TiO_2 demonstrated higher concentrations of titanium relative to 20 controls in which levels were below detectable limits (56). Cadmium concentrations in EBC provided by those working in the airport apron area was higher relative to office workers (24).

These studies of occupationally-exposed workers confirm significant exposure to specific metals. The cohorts used are small, reflect uncommon conditions or exposures, and document data that may not be applicable to larger groups. To document an occupational exposure, it may be more efficient to measure filters collected for environmental monitoring rather than quantify EBC metals concentrations. In addition, the lung is not a passive filter but actively metabolizes and transports metals. The EBC metals concentrations should not automatically be interpreted to support a participation, or a lack of a participation, in an observed biological effect.

Conclusion and recommendations

The results of investigation demonstrate that transition and post-transition metals can be detectable in EBC. Currently, the measurement of metals concentrations in EBC is a research tool in the investigation of the participation of metals in the pathogenesis of disease and injury following an exposure. Measurements of EBC metals may be especially valuable in research to define associations between human disease and smoking. However, the considerable dilution of the EBC sample currently limits which metals can be accurately quantified; those which are normally measurable currently include iron, zinc, and copper. Individuals with a specific history of occupational and environmental exposures to metal-abundant particles are exceptions with additional metals being measurable. Furthermore, failure to measure and address variability in the dilution of airway aerosols in EBC poses limitations to analysis. Measurements of EBC metals concentrations is also a potentially effective research technique in delineating translocation of metals from the lung.

Quantification of EBC metals concentrations is currently not reliable for clinical use in either supporting or determining any diagnosis. Issues that must be addressed prior to increased use of EBC metals measurements include the establishment of both standardized collection and measurement techniques. After this is achieved, reference values for metals in EBC collected from healthy normal populations can be compiled.

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Table 1.

Concentrations of metal in lavage collected from healthy subjects

Study	n	Subjects	Smoking status	Fe	Zn	Cu	Cr	Mn	Ni	Pb	Ti
Sabbioni et al, 1987	4–22	Not provided	Not provided	508	510	215	10	2.7	145	34.6	<1
Romeo et al., 1992	25	Patients	N	106			0.6	1.5		3.2	
Corhay et al, 1995	45	Healthy	N and S	170	300	50	20		10		140
Nelson et al., 1996	21	Healthy	N and S	2–9							
Harlyk et al, 1997	157	Patients	Not provided	0–120	10–230	0–15					
Stites et al., 1999	8	Healthy	N	0							
	8	Healthy	S	100							
Ghio et al., 1998	22	Healthy	N	40–70							
Ghio et al., 2003	28	Healthy	N	≈50							
Bargagli et al., 2008	9	Healthy	N and S	32	8	3	5	10	3	10	
Ghio et al., 2013	20	Healthy	N	<50							

N is non-smoker and S is smoker. Metal concentrations are provided in ppb μg/L).

Table 2.

Concentrations of metal in EBC collected from healthy subjects

Study	n	Subjects	Smoking status	Fe	Zn	Cu	Cr	Mn	Ni	Pb	Ti
Caglieri et al., 2006	25	Healthy	N and EX				0.28				
Mutti et al., 2006	50	Not provided	N and EX	≈10		≈1.25		≈0.1	<1	<0.10	
Goldoni et al., 2008	20	Healthy	N				0.18				
Corradi et al., 2009	33	Healthy	N	1.20	1.60	0.60	ND	0.10	0.02	0.02	
Vlasic et al., 2009	22	Healthy	N	22							
Fox et al., 2013	8	Not provided	Not provided				0.25	0.57	0.87		
Hulo et al., 2014	16	Not provided	N and S	0.50				0.32	0.24		
Pelclova et al., 2015	20	Not provided	N, S, and EX								ND
Leese et al., 2017	22	Not provided	Not provided				0.01–0.09				

Metal concentrations are provided in ppb µg/L). N is non-smoker, S is smoker, and EX is ex-smoker. ND is not detectable.