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Trace metals in fluids lining the respiratory system of patients with idiopathic pulmonary fibrosis and diffuse lung diseases

Elena Bargagli[a,*], Federico Lavorini[a], Massimo Pistolesi[a], Elisabetta Rosi[a], Antje Prasse[b], Emilia Rota[c], Luca Voltolini[d]

[a] Section of Respiratory Medicine, AOUC, Florence, Italy
[b] Hannover Medical School, Clinic for Pneumology, Hannover, Germany
[c] Environmental Sciences Siena University, Siena, Italy
[d] Thoracic Surgery Unit, University Hospital Careggi, Largo Brambilla, 1, 50134, Florence, Italy

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is an interstitial lung disease with a poor prognosis and an undefined etiopathogenesis. Oxidative stress contributes to alveolar injury and fibrosis development and, because transition metals are essential to the functioning of most proteins involved in redox reactions, a better knowledge of metal concentrations and metabolism in the respiratory system of IPF patients may provide a valuable complementary approach to prevent and manage a disease which is often misdiagnosed or diagnosed in later stages.

The present review summarizes and discusses literature data on the elemental composition of bronchoalveolar lavage (BAL), induced sputum and exhaled breath condensate (EBC) from patients affected by IPF and healthy subjects. Available data are scanty and the lack of consistent methods for the collection and analysis of lung and airways lining fluids makes it difficult to compare the results of different studies. However, the elemental composition of BAL samples from IPF patients seems to have a specific profile that can be distinguished from that of patients with other interstitial lung diseases (ILD) or control subjects. Suggestions are given towards standard sampling and analytical procedures of BAL samples, in the aim to assess typical element concentration patterns and their potential role as biomarkers of IPF.

1. Introduction

During biomolecular evolution, several elements such as Cr, Co, Cu, Fe, I, Mn, Mo, Ni, Se or Zn were selected to carry out a wide range of biological functions and traces of them (ng/g or μg/g) became essential for cell metabolism, including the activation or inhibition of enzymatic reactions, and the regulation of gene and membrane functions. All living organisms and tissues require the intake of essential elements in proper proportions: an excess, deficiency, or imbalance may disturb the cell functions and may seriously affect health [1,2]. Risks of developing adverse health effects are usually evaluated indirectly, by biomonitoring the dietary intake and/or the inhaled amounts of airborne trace elements. However, determining actual element concentrations in biological samples such as blood, urine, feces, bone, or cerebrospinal fluid has the advantage to better reflect their bioavailability and the amounts that can reach target tissues.

During the last decades, knowledge of metal transport proteins (including metallothioneins) and the transfer of their different chemical forms to different organs and tissues has significantly improved [3,4]. Unlike other organs, lungs are directly and continuously exposed to high oxygen concentrations, exogenous oxidants and pollutants; thus, they have the greatest susceptibility to oxidative stress and pollutant toxicity, from which they protect themselves through the action of own constitutive and inducible antioxidants and detoxification mechanisms [5]. The biological effects of inhaled metals occur at the sites of first contact and it is therefore necessary to study the metabolism and impact of essential and toxic metals in the respiratory system rather than by traditional exposure biomarkers such as blood or urine. Among

Abbreviations: IPF, idiopathic pulmonary fibrosis; ILD, interstitial lung diseases; COPD, chronic obstructive pulmonary diseases; CF, cystic fibrosis; PLCH, pulmonary Langerhans cell histiocytosis; BAL, bronchoalveolar lavage; EBC, exhaled breath condensate; TLC, total lung capacity; ROS, reactive oxygen species; SOD, superoxide dismutase; DMT1, divalent metal transporter 1; GPX, glutathione peroxidase; ETAS, Selectrothermal atomic absorption spectrophotometry; ICP, inductively coupled plasma spectroscopy; OES, optical emission spectroscopy; MS, mass spectrometry; NAA, neutron activation analysis; MS, mass spectrometry

* Corresponding author. AOUC Careggi University Hospital, Respiratory Medicine Unit, 50121 Florence, Italy.
E-mail address: bargagli2@gmail.com (E. Bargagli).

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essential trace elements, the understanding of Fe metabolism is the most advanced, and studies on lung physiology, dysfunction and injury usually consider only this metal [6,7]. Iron plays a fundamental role in the respiratory chain, as reactive forms of the metal are accumulated in the lung for cellular growth and proliferation, and decreased intracellular Fe concentrations suppress the generation of epithelial cell surface plasmin, which is essential for repairing damaged lung tissue [8]. However, if not appropriately chelated, Fe can promote the formation of harmful free radicals. Many other trace elements are involved in the regulation of Fe metabolism and contribute to the functioning and protection of the lung. Thus, a better knowledge of the occurrence and distribution of trace elements in the respiratory system and changes occurring during lung diseases could give new insights for diagnostic, therapeutic and preventive actions, especially for severe and complex diseases such as idiopathic pulmonary fibrosis (IPF).

IPF is a complex interstitial lung disease of unknown etiology, characterized by severe and progressive fibrosis of the alveolar interstitium with histopathological or radiological patterns typical of usual interstitial pneumonia. Probably, it is the result of complex interactions between genetic and environmental factors [9,10]. Several infectious, occupational and toxic etiologies have been suggested and epidemiological studies have shown associations between IPF development and cigarette smoke, wood and metal dust, silica and other inhaled environmental agents as well as co-morbidities with gastroesophageal reflux and type 2 diabetes [11,12]. In general, it is believed that IPF results from the aberrant activation of injured alveolar epithelial cells producing mediators that promote the proliferation of fibroblasts and fibrocyte recruitment with the formation of myofibroblastic foci, the accumulation of excessive extracellular matrix and lung remodeling [13–15]. Although barely investigated, the assessment of trace element distribution in the lung tissue and lining fluids could probably be an useful prognostic/predictive tool providing additional and complementary information with respect to genetic, proteomic, or molecular biology approaches. The chemical analysis of lung tissue samples is the most realistic way to assess the elemental composition of lung, but this invasive approach is rarely used for the diagnosis of IPF. Through an up-to-date literature survey of data on the elemental composition of airway secretions and breath condensates, this paper aims at providing an overview of available knowledge on trace element concentrations in IPF patients and an evaluation of their potential role as biomarkers of the disease.

2. Trace elements in BAL samples

Bronchoalveolar lavage (BAL) is a rather simple method to obtain a biological matrix for histopathological and chemical analysis. Although the chemical composition of this non-homogenous matrix can be contaminated by element contribution from the saline solution and the bronchoscope, working with BAL samples, instead of small lung biopsies, increases the sample representativeness and as a rule, reduces contamination by blood elements. Surveys on the elemental composition of BAL began 30 years ago [16,17] and although already in 1987 Sabbioni et al. [18] investigated on what should be the most suitable technique to analyze samples from differently exposed workers and the composition of the saline solution used for bronchoalveolar lavage, standardized methods for the collection and analysis of BAL are still lacking. Due to the inhomogeneous nature of samples, some authors [19] suggested that the amount of a trace element per 1000 macrophages should be the best way to establish baseline concentrations in BAL; on the contrary, other authors [20] found that concentrations (μg/l) of several elements in BAL were unrelated to age, sex, recovered volume of fluid, as well as to the total number of cells and alveolar macrophages. Thus, element concentrations in BAL have been expressed in different ways (mass/volume, mole/volume or on the basis of total cell number or number of macrophages), and for a given element available data are variable and difficult to compare. Harlik et al. [21] analyzed Zn, Cu and Fe concentrations (expressed in μg/kg) in 157 whole samples of BAL obtained for other diagnostic purposes. The supernatant was separated from cells and solid particles by centrifugation. The results showed that metal concentrations in the whole sample and the supernatant were linearly correlated and almost all elements (about 90%) were in the supernatant. With few exceptions, the increase of Cu concentrations was associated to a decrease of Zn content, and low Fe concentrations were found in a few BAL samples with high Zn levels. Analytical determinations were performed by atomic absorption spectrophotometry without any pre-treatment and in most samples the concentrations of Cu, Fe and Zn were < 15, 120 and 200 μg/kg, respectively. However, as shown in Table 1 for BAL samples from healthy controls, the results of analytical determinations are affected by pre-treatment methods, and their complete chemical digestion in a Teflon bomb or irradiation followed by NAA analysis [17,18] give the highest values. Since the study by Sabbioni et al. [18] it was known that the elemental composition of BAL changes among subjects working in different industries as well as between control groups and patients affected by sarcoidosis. Thus, reliable comparisons of the elemental composition of BAL can only be made among samples collected within homogeneous groups of persons (e.g. patients with the same disease, workers with the same occupational exposure), pretreated and analyzed with the same procedures. To evaluate if data on BAL elemental composition, integrated with those from clinical examinations, can be used as a diagnostic tool for lung diseases Bargagli et al. [22] compared Cd, Cr, Cu, Fe, Mn, Ni, Pb, V, and Zn concentrations in BAL samples from control subjects and from patients with pulmonary Langerhans cell histiocytosis (PLCH), sarcoidosis, and IPF. Among the analyzed elements, the concentrations of Cd and V were below detection limits; those of the other elements varied widely and some outlier values were also found, especially for Cu and Mn. BAL from IPF patients showed significantly lower median concentrations of Cr, Mn, Ni and Zn and a slightly higher Fe content than control subjects. Results expressed in ng/macrophages showed the highest Fe content and the lowest Mn and Zn contents in samples from IPF patients (Table 3) [22].

Table 1
Mean concentrations of Cu, Fe, and Zn (μg/l; ± SD) in whole BAL samples from control subjects, following different pre-treatments and analytical methods.

Metal	(μg/l)	Sample pre-treatment	Analytical technique[a]	References
Cu	215 ± 122	dissolved in Teflon bomb	ETASS/ICP	[18]
	2.81 ± 3.02	slight acidification with HCl	ETASS	[22]
Fe	758	Irradiation	NAA	[17]
	508 ± 204	Irradiation	NAA	[18]
	32.3 ± 26.1	slight acidification with HCl	ETASS	[22]
Zn	695	Irradiation	NAA	[17]
	510 ± 120	Irradiation	NAA	[18]
	8.21 ± 4.31	slight acidification with HCl	ETASS	[22]

[a] ETASS: Electrothermal Atomic Absorption Spectrophometry; ICP: Inductively Coupled Plasma Spectroscopy; NAA: Neutron Activation Analysis.

3. The elemental composition of induced sputum

Induced sputum is a heterogeneous matrix obtained by inducing expectoration through the inhalation of nebulized hypertonic saline. This approach is less invasive than bronchoscopy and BAL and allows the collection of a fluid phase with a mixture of cells and solutes which are considered representative of the larger airways. If in the clinical practice the differential cell count in BAL is widely accepted and recommended as a diagnostic/prognostic tool for sarcoidosis, PLCH, IPF and other ILD [23,24], the cell count and FeNO values in induced

Table 2
Concentrations of Cu, Fe, Mn and Zn (μg/l; range and median value) in induced sputum (supernatant), and EBC samples from healthy adult subjects, and 'normal' values (in μg/g, dry weight) in mineralized lung tissues from living donors.

	Cu	Fe	Mn	Zn	References
Ind. sputum	8.6 (3.0-16.4)	13.5 8.6–21.5	0 0–0.25	15.3 (10.4–25.6)	[26]
EBC	0.60 (0.30-1.80)	1.20 (0.25-6.00)	0.1 (0.04–0.25)	1.6 (0.50–22.0)	[37]
Lung tissue	6.02 2.2-40.6	745.6 (201–2979)	0.62 (0.14–7.77)	49.4 (1.13–338)	[38]

Table 3
Metal concentrations (μg l^{-1}; average ± SD of total metal concentrations) in BAL from patients affected by diffuse pulmonary diseases (references 22,25).

	Patients
Cr	2.69 ± 0.86
Cu	4.54 ± 4.50
Fe	24.2 ± 13.9
Mn	5.39 ± 8.82
Ni	1.70 ± 1.41
Pb	10.1 ± 17.1
Zn	6.01 ± 3.37

sputum may serve as valuable additional tools in obstructive lung diseases [25]. Gray et al. [26] analyzed Cu, Fe, Mn and Zn concentrations in sample of induced sputum from patients with cystic fibrosis (CF), non-CF bronchiectasis, asthma, COPD, and 20 healthy controls. Element concentrations were determined by ICP-OES and the results indicated a statistically significant increase of Zn and Fe concentrations in CF and non-CF bronchiectasic patients versus controls. The major weakness of this method is that it is impossible to establish if elements in the sputum originate from injured lung tissue or from luminal inflammatory cells. Furthermore, other possible sources of elements such as micro-vascular leakage, cell membrane channelopathies or contamination by inhaled particulates, cannot be disregarded. As the elemental composition of expectorated sputum can be affected by oropharyngeal contamination, this matrix seems less suitable than BAL to assess the pattern of element concentrations in IPF patients, but, to our knowledge, the issue has never been approached in the literature.

4. Elements in exhaled breath condensate

Exhaled breath consists of a gaseous phase with many volatile compounds and a liquid phase containing aerosol particles with slightly volatile and nonvolatile compounds. Some breath volatile compounds have a distinctive smell that has been used as indicator of several diseases since the time of Hippocrates. Dogs can discriminate exhaled breath samples from subjects with and without lung cancer [27] and very sensitive electronic noses are now available for smellprint identification [28]. If identification of volatile substances, particularly NO, was the main objective of breath studies, in recent years a growing interest has been devoted to the analysis of exhaled breath condensate (EBC). During normal tidal breathing, small aerosol droplets (< 0.3 μm) join the vapor stream passing over the mucus layer lining the lung and airways, and the droplets can be collected through the cooling of the exhaled breath [29,30]. Most of the condensate consists of water vapor, but also contains non volatile compounds such as inflammatory mediators, and trace elements. Like the analysis of BAL or induced sputum, investigating EBC samples can provide useful information on lung pathobiology and on airways and lung exposure to airborne persistent pollutants in occupational and environmental settings [31,32]. This absolutely non-invasive approach allows to study patients of any age, even at home. Main concerns on determining the elemental composition of EBC are the lack of standardized procedures for the collection of representative samples and risk of their contamination by elements from saliva and sampling devices. Most available data on the elemental composition of EBC refer to occupationally exposed workers [30,32–35]. Some authors [36,37] analyzed concentrations of serum pneumoproteins and several elements in EBC samples from asymptomatic smokers and patients with obstructive pulmonary diseases (including COPD and asthma). In COPD patients the EBC content of Fe and Cu was lower than in control nonsmokers and Cu levels positively correlated with values of the forced expiratory volume (FEV_1). Comparisons between concentrations of essential trace elements and toxic metals in EBC samples from 33 healthy subjects, 22 patients with sarcoidosis, 15 with non-specific interstitial pneumonia and 19 with IPF showed only small overall differences among different groups of patients [37]. However, in EBC samples from patients with interstitial lung diseases of unknown etiology (all non-smokers or ex-smokers), the concentrations of Ni, Si and Cr were significantly higher and those of Fe, Cu, and Se significantly lower than in EBC from healthy non-smokers. In particular, IPF patients also showed a lower EBC content of Mo than controls and a significant relationship between Fe concentrations in EBC and total lung capacity (TLC; %). Average EBC concentrations of Cu, Fe and Zn (0.10, 0.30, and 2.1 μg/l, respectively) were much lower than those in BAL samples from IPF patients. Moreover, in IPF patients the BAL samples had significantly lower ($p < 0.01$) median concentrations of Cr, Mn, Ni and Zn than controls [22], whereas in EBC samples [37] the content of these elements was higher or in the same range of those in healthy non-smoker subjects.

To evaluate the potential role of the elemental composition of induced sputum and EBC in giving insight into respiratory disorders such as IPF, it is mandatory to define normal element concentrations in samples from healthy adult subjects. However, as shown for BAL in Table 1, available literature data for these two biological matrices in control groups are highly variable (Table 2). Median values of Cu, Fe and Zn concentrations in the supernatant of induced sputum are an order of magnitude higher than that in EBC samples, but, element concentrations show the same pattern: Zn > Fe > Cu > Mn. In BAL supernatant Fe concentrations are four times higher than those of Zn (Table 1) and a much higher Fe/Zn ratio (about 15) has recently been reported [38] for the 'normal' composition of lung tissue (Table 2). Mumby et al. [39] determined the content of pro-oxidant Fe in both BAL and EBC of patients undergoing cardiopulmonary bypass surgery and found in all samples a decreasing trend of values pre- to post-surgery. However, no correlation was found between the pro-oxidant Fe levels of the two matrices and it was supposed that this discrepancy was due to different areas of the respiratory system sampled with BAL and EBC.

5. Oxidative stress and IPF

It is currently thought that the development of IPF is due to persistent stimuli or injury followed by abnormal reepithelialization and dysregulated remodeling of the extracellular matrix and several lines of evidence in animal models and patients indicate the involvement of oxidative stress in alveolar injury, inflammation and fibrosis development [40–42]. Increased concentrations of lipid peroxidation products, oxidized proteins and an altered antioxidant enzyme status or the depletion of glutathione, the most abundant low-molecular-weight antioxidant, have often been reported in epithelial lining fluid of IPF patients [43–47]. Moreover, serum levels of reactive oxygen species (ROS) negatively correlate with pulmonary function in IPF and may predict disease severity [48]. Transition metals are essential for the function of most proteins involved in redox reactions, and when mechanisms regulating their metabolism (uptake, transport, storage

and excretion) are altered, they can bind to different protein sites or be replaced by other elements at their natural binding sites [49]. Some metal ions and especially Fe^{2+} and Cu^{2+} are involved in enzymes participating in redox reactions and in the conversion of active O_2-containing compounds, whereas other ions such as Ca^{2+} and Zn^{2+} are targeted to other enzymes or transcription factors and behave as non-redox ions [49]. There is evidence of a disordered Fe homeostasis in IPF patients. Kim et al. [6], for instance, observed Fe deposition in association with alveolar septal capillary density and pulmonary hypertension in non-fibrotic lung tissue (from biopsies or explants) from 149 IPF patients. Puxeddu et al. [50] analyzed total Fe concentrations in BAL samples from 47 IPF patients and 14 healthy controls (the BAL supernatant was mineralized with 15 µl of HNO_3 0.2% and the results of analytical determinations were normalized to the volume of epithelial lining fluid recovered by lavage, using urea as marker of dilution) [51]. Total Fe concentrations were significantly higher in never-smoking or ever-smoking IPF patients than in never-smoking healthy controls and were in the same range as in healthy current smokers. However, no relationship was found between Fe accumulation in BAL from IPF patients and their tobacco smoke history. Cell counts showed a significantly higher number of Fe-laden alveolar macrophages and macrophage hemosiderin accumulation in IPF BAL samples than in controls. Hemosiderin content was significantly higher in patients with elevated artery pressure, likely indicating vascular damage and pulmonary veno-occlusive diseases [50]. However, Fe metabolism and homeostasis can be altered by acute or chronic lung injury as well as by changes in O_2 availability (hypoxia and hyperoxia) [7,52]. Lungs are the site of massive O_2 exchange and have large blood supplies and peculiar molecular mechanisms for the regulation of Fe metabolism and the balance between oxidants and antioxidants [7]. For instance, systemic Fe-deficiencies are characterized by low Fe concentrations in serum and liver, but not in the lung [53]. Upon Fe exposure, concentrations of transferrin, which imports Fe from the airways into the lung epithelial cells and exports the metal to the outside, increases in serum, but not in the pulmonary system [54]. The lung can probably regulate Fe metabolism through transferrin receptors located in the bronchial epithelium, macrophages, and lymphoid tissues. Unlike the intestinal Divalent Metal Transporter 1 (DMT1), the transcripts of this Fe binding protein in airways and alveolar epithelium do not appear to be highly regulated by the Fe status [54,57].

6. Metabolism of transition metals in the lung

Understanding metal metabolism in the lung and airways is complicated by peculiar and partly unknown mechanisms involved in the redox balance in an environment affected by the concomitant impact of inhaled elements and those arising from vascular leak or released by necrotic airway cells or bacteria. Inhaled metals originate from a number of external sources and differ greatly in their physico-chemical characteristics and bioavailability. Anyhow, when compared to the elemental composition of BAL from control subjects, samples from IPF patients show significantly lower Cr, Mn, Ni and Zn concentrations and slightly higher Fe and Cu concentrations (expressed as µg/l; ng/cellx10^6 or ng/macrophages) [22]. Iron and Cu produce in vivo hydroxyl radicals and this pattern of element concentrations in IPF patients likely indicate the presence of free radicals and oxidative stress. Iron is the most versatile cofactor in biological redox reactions and when not appropriately chelated it can promote the formation of harmful free radicals [55,56]. Copper is an essential co-factor for oxidation-reduction reactions and an extracellular Cu-containing SOD (EC-SOD) occurs in the lung; Cu is also involved in the maintenance of Fe homeostasis. To prevent an excessive production of ROS, several Cu chaperones regulate the cellular uptake and metabolism of the metal. Copper deficiency can compromise (directly or indirectly) the oxidant defense system by decreasing the activity of Cu/Zn-SOD, ceruloplasmin, catalase, Se-dependent glutathione peroxidase (Se-GPX), and ROS scavengers such as metallothioneins or glutathione [58]. Furthermore, the reduced activity of Cu/Zn-SOD can promote the formation of peroxynitrite, a potent initiator of oxidative damage, by decreasing the content of bioactive NO which plays an important role in the regulation of the vascular tone [59]. Manganese is another element promoting the production of antioxidants (i.e., the mitochondrially expressed SOD) and ROS. It is chemically and structurally similar to Fe, however, Mn^{2+} ions are more stable than Fe^{2+} ions in water solutions and are less likely to undergo a spontaneous redox cycling. Iron and Mn share many protein transporters such as DMT1 and as a rule, during low Fe bioavailability there is a Mn accumulation; conversely, altered Mn uptake or metabolism can disrupt the homeostasis of Fe and other transition metals [60]. However, Fe deficiency in the lung does not enhances the pulmonary uptake of Mn and Fe and Mn absorption seem to be mediated through different mechanisms [60]. Among analyzed element in BAL from IPF patients [22], Fe/Mn concentration ratio was three times higher than in samples from control subjects. Probably, the decrease of Mn concentrations was due to the oxidative stress and the disruption of transition metal homeostasis. Moreover, the three SODs occurring in the lung (cytosolic Cu/Zn, mitochondrial Mn, extracellular) have specific functions and the Mn-SOD is induced during acute inflammatory stages of the lung parenchyma, but its antioxidant defense is probably impaired during the progression of fibrogenesis [61]. Chromium (VI) is carcinogenic, however in the lung it is efficiently reduced to Cr (III) by ascorbate, glutathione or cysteine [62] and these reactions can produce different ROS species [63]. Although Cr (III) competes with Fe for a binding site in transferrin, Cr supplementation does not seem to affect Fe nutritional status [64]. Nickel is another potentially toxic metal and much of its toxicity is associated with interferences with the metabolism and physiological processes of Mn, Zn, Ca and Mg. Chronic inhalation exposure to Ni dusts and aerosols contributes to respiratory diseases (asthma, bronchitis, sinusitis and pneumoconiosis), and although lower concentrations were measured in BAL from IPF patients than in control subjects, it is known that Ni can cause formation of free radicals in both human and animals with modifications to DNA bases, enhanced lipid peroxidation and altered Ca and sulphydryl homeostasis [65].

Zinc is a component of more than 300 enzymes with catalytic, structural and regulatory functions and is involved in numerous aspects of cellular metabolism. Zinc acts as an antioxidant by protecting sulphydryl groups of proteins against free radical attack or by reducing the formation of free radicals through the replacement of Cu or other redox-active metals from their binding site [66]. It can reduce the formation of free radicals through the inhibition of the NADPH oxidase, the induction of metallothioneins or the formation of the Cu/Zn-SOD, where Cu provides the catalytic activity and Zn plays a critical structural role. Through the up-regulation of a Zn-finger protein (A20) and the reduced production of inflammatory cytokines, Zn behaves also as an anti-inflammatory agent [67]. Macrophages (M2 phenotype) produce high levels of H_2O_2 and He et al. [68] showed that Cu/Zn-SOD polarizes macrophages to this phenotype making the generation of H_2O_2 generation in alveolar macrophages a critical factor in the pathogenesis of pulmonary fibrosis. In IPF patients Cu/Zn-SOD and Mn-SOD are upregulated whereas EC-SOD is practically absent in fibrotic area and fibrotic foci, suggesting its depletion in places where the oxidative burst is particularly strong [46]. The structural remodeling of lung tissues is mediated by metalloproteases and in BAL of IPF patients there is an increase in different proteases, especially matrix metalloproteinases-7 (or matrilysin), which contributes to lung epithelial damage [46,69]. Considering that most metalloproteases require Zn as divalent cations and that many other transition metals are involved in the oxidative stress and protease/antiprotease imbalance, a better knowledge of metal occurrence and metabolism in the lung of IPF patients will be very useful for an improved diagnosis, therapeutic and preventive actions against a disease associated with high morbidity and mortality. Selenium is a metalloid with properties of both a metal and a

non- metal, it seems advisable to analyze this element in future studies because Se-glutathione peroxidases seems highly efficient in defense against oxidative stress [70].

7. Research perspectives and conclusions

Although available data on trace metal concentrations in BAL, induced sputum and EBC appear scanty and highly variable, this survey indicates that the chemical composition of BAL would probably help to differentiate the IPF pattern from that of other pulmonary diseases and/or healthy control subjects. The element concentration pattern both in sputum and EBC (Zn > Fe > Cu > Mn) is different from those reported for BAL and lung tissue (Fe > Zn > Cu) and does not support current thoughts about the involvement of oxidative stress in alveolar injury and IPF pathogenesis. The increase in free radicals is associated to a disordered homeostasis of Fe as shown by increased concentrations in BAL of IPF patients, a higher number of Fe-laden alveolar macrophages and greater macrophage hemosiderin accumulation than in controls. Increased Fe deposition has been detected in lung biopsies from 149 IPF patients. The increase in free radicals and oxidative stress are likely responsible for the significant decrease of Zn, Mn, and Cr concentrations in BAL samples. Zinc is involved in numerous aspects of cellular metabolism, acts as an antioxidant and is involved in the structural remodelling of lung tissue through metalloproteases. Manganese also promotes the production of antioxidants such as the mitochondrially expressed Mn-SOD, which is induced by acute inflammatory stages of the lung parenchyma, although its antioxidant defense is probably impaired during the progression of fibrogenesis. Chromium is not a true antioxidant, however Cr (III) competes with Fe for a binding site in transferrin. Thus, concentrations of transition metals in BAL from IPF patients can likely play a role, complementary to traditional biological approaches, as a prognostic/predictive tool. However, available literature data are limited and further studies on BAL samples from a larger population of IPF patients and controls are needed to establish reference values and typical patterns of element concentrations. Reliable comparisons among results of different studies were the main problem in reviewing the available literature on the elemental composition of BAL. Future research will require standardized methodology for the pre-treatment and analysis of samples, and the interpretation of results for specific groups of patients and control subjects in relation to data from their clinical examination and diagnosis, lifestyle, occupational and environmental settings.

BAL is a complex, non-homogenous matrix and reliable assessment of its chemical composition is also hampered by possible contamination of samples by the saline solution and blood or elements released by the bronchoscope, disposable syringes and test-tubes. Direct determination by sensitive analytical methods and limited sample handling (to reduce the risks of contamination) provided reliable and accurate assays of trace element concentrations in BAL samples. Whenever possible, it seems advisable to collect BAL samples from subjects that are not under pharmacological treatments and without concomitant pathologies. In order to standardize the procedure and to evaluate concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn in broncho alveolar lavage it is important to kept refrigerated at −20 °C and slightly acidified with HCl for example before analysis by atomic absorption spectrophotometry. Representative BAL sub-samples can be therefore stored and cells should be separated from the supernatant. The latter can be analyzed for trace element concentrations by different techniques (ETAAS, ICP-MS, ICP-OES or NAA); however, to obtain reliable comparisons among samples, it seems necessary to analyze always total concentrations of elements through a complete mineralization of samples. BAL samples must be colorless to exclude contamination by elements in the blood; a suitable Standard Reference Material and the saline solution used for instillation must be digested and analyzed together with BAL samples. Although most studies on the chemical composition of BAL have only considered Fe, Cu, and Zn concentrations, it seems necessary to analyze other elements playing a role in the IPF development such as Ca, Co, Cr, Mg, Mo, Ni, and Se. Together with the chemical analyses of the supernatant, in BAL subsamples it is necessary to perform differential cell counts and enzyme assays in the fluid fraction. By such comprehensive approach, applied to a large population of patients and controls, it will possible to establish the role of the elemental composition of BAL as prognostic biomarker of IPF.

Conflicts of interest

The author has no conflict of interest to declare.

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