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ORIGINAL ARTICLE

Erythrocyte osmotic resistance recovery after lung volume reduction surgery

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Abstract

OBJECTIVES: Alteration of erythrocyte osmotic resistance, with increment of reticulocytes, is common in emphysema. This fragility is probably due to an altered fatty acid membrane composition from lipid peroxidation, a reaction triggered by the disease-related increment of reactive oxidative species. We analysed the effects of lung volume reduction surgery (LVRS) on this anomaly compared with respiratory rehabilitation (RR) therapy.

METHODS: We retrospectively compared 58 male patients with moderate-to-severe emphysema who underwent LVRS with 56 similar patients who underwent standardized RR. Respiratory function parameters, erythrocyte osmotic resistance and antioxidant enzymes levels were evaluated before and 6 months after treatments.

RESULTS: Significant improvements in respiratory function, exercise capacity, unsaturated fatty acid content (+10.0%, $P = 0.035$), erythrocyte osmotic resistance (hyperosmolar resistance −21.0%, $P = 0.001$; hyposmolar resistance −18.0%, $P = 0.007$) and erythrocyte antioxidant enzymes [superoxide dismutase (SOD) +60.0%, $P < 0.001$; glutathione peroxidase +39.0%, $P = 0.004$ and glutathione reductase +24.5%, $P = 0.008$] were observed after surgery. In the RR group, we did not find any significant improvements in osmotic resistance, although respiratory and functional parameters were significantly improved. Correlation analysis in the surgical group showed that the reduction in residual volume (RV) significantly correlated the normalization of hyperosmotic ($P = 0.019$) and hyposmotic resistances ($P = 0.006$), the decrease in the absolute number of reticulocytes ($P = 0.037$) and increase in SOD ($P < 0.001$).

CONCLUSIONS: LVRS improved unsaturated fatty acid content, erythrocyte osmotic resistance and levels of erythrocyte antioxidant enzymes compared with RR. Correlations between erythrocyte osmotic resistance and antioxidant intracellular enzymes with RV suggest that reduction in lung hyperinflation with the elimination of inflammatory emphysematous tissue may explain such improvements after surgery.

Keywords: Emphysema • Lung volume reduction surgery • Erythrocyte osmotic resistance • Oxidative stress

INTRODUCTION

Pulmonary emphysema is characterized by chronic inflammation with typical alterations in the lung as well as other systemic disturbances mainly originating from an up-regulation of endogenous oxidant–antioxidant balance [1, 2]. The overproduction of reactive oxidant species (ROS) increases the consumption of the related antioxidant intracellular enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) [3–5]. ROS hyperactivity activates the peroxidation of the poly-unsaturated fatty acids, which constitute the structure of the cellular membrane [6]. The resistance of the erythrocyte cell wall to the variation of the osmotic stress is reduced [7]. Haemolysis is favoured by the decrement of circulating haemoglobin (Hb), thus aggravating hypoxia and stimulating erythropoietin secretion [8].

In selected patients, lung volume reduction surgery (LVRS) has shown improvement in respiratory function and quality of life more significantly and longer-lasting than maximal medical therapy and respiratory rehabilitation (RR) [9, 10], with regularization of many altered pathways [11–14].

We hypothesized that the reduction in the inflammatory status after LVRS might have an effect on oxidative stress with a rearrangement of the fatty acid profile of the membrane and restoration of normal osmotic resistance. Here, we analysed these changes in operated patients when comparing them with a homogeneous group of emphysematous patients undergoing RR.

MATERIALS AND METHODS

In 1995, one of us (Tommaso Claudio Mineo) proposed the research project entitled 'Pulmonary emphysema research group', aimed at

clinical investigations into LVRS. After the approval by the Research Ethics Board of our academic institution, a dedicated 'Emphysema Center' was setup shortly thereafter, through which a large population of emphysema patients were studied and a total of 280 have undergone lung volume reduction procedures until now. This strong effort involved a dedicated group of investigators generating a number of publications [11–13]. The current study is an integrated part of this programme and approved by our Institutional Ethics Review Board (prot. no. PTV0023/2005).

Study design and population

This study was a retrospective non-randomized trial involving two groups of male patients with moderate-to-severe emphysema. Fifty-eight consecutive patients who underwent standard videothoracoscopic LVRS (LVRS group) without postoperative RR between January 2005 and December 2009 were compared with 56 similar patients eligible for surgery who as voluntaries preferred a standardized RR programme (RR group) after having denied their consent to the operation. Crossing over from one group to another was not allowed for the first 6-month period after the treatment. All patients released full informed consent to study enrolment.

The analysis included intra- (baseline vs 6 months post-treatment) and inter-group (LVRS group vs RR group) evaluations. The observational period was set at 6 months post-treatment, assuming that as the optimal period to reach the maximal improvement for both treatments.

All patients in the three groups were clinically stable, no smokers for 3 years and free of any hematological disorder. Furthermore, they were performing regular mild physical activity and were receiving an adequate balanced diet (1800 kcal/day). All emphysematous patients were receiving an inhaled steroid and β_2-agonist therapy. In the 6 months prior to study enrolment, none of them started oral steroid, *N*-acetyl-cysteine or any other drug potentially interfering with erythrocyte osmotic resistance or oxidative stress. We excluded those patients who were receiving long-term oxygen therapy or had concomitant endocrine, metabolic, cardiovascular or other chronic diseases.

Respiratory evaluations

Respiratory assessments included timed spirometry and plethysmography, with single-breath diffusing lung capacity for carbon monoxide (Vmax22; Sensor Medics, Yorba Linda, CA, USA) and arterial blood gas analysis (GEM Premier 3000; Instrumentation Laboratory, Lexington, MA, USA). Exercise tolerance was assessed with the standard 6-minute walk test. Dyspnoea index was rated with the Medical Research Council (best = 1 and worst = 3). Quality of life was assessed with the St. George Respiratory Questionnaire general score (best = 0 and worst = 100) [15].

Surgical intervention

Four-port, video-assisted thoracoscopic LVRS was performed in all surgical interventions, as elsewhere described [11]. The most damaged portions of the lung were re-evaluated by intraoperative inspection and resected using simple non-buttressed suture lines, possibly excising a single strip of parenchyma to reduce the lung volume by ~30% [11].

Rehabilitation therapy

Our standard RR programme required supervised sessions of 3 hours, 5 days per week for at least 6 weeks. The first half of each session included educational activity, such as breathing retraining, chest clearance, energy conservation, nutritional and medication education and psychosocial support, whereas the second half included physical conditioning with inspiration resistive exercises and upper and lower extremity training.

Blood sample assay

Blood samples were collected between 6:00 and 7:00 am into Vacutainer® tubes (Becton Dickinson Ltd., Cowley, Oxford, UK) containing tripotassium ethylenediaminetetraacetic acid as anticoagulant, at a concentration of 1–5 mg/ml.

Erythrocytes were separated by centrifugation (2000 rpm for 10 min, ALC 4232 centrifuge) and stored at −70°C in isotonic buffer until used for membrane purification. Cell membranes (ghosts) were prepared by lysis with hypotonic buffer (phosphate 5 mM, pH 8, ethylenediaminetetraacetic acid 0.5 mM), precipitated by centrifugation and washed several times to eliminate Hb residues. Ghost lipids were extracted with chloroform/methanol and fractionated by silicic acid chromatography (200–400 mesh BIORAD, CA, USA) into non-polar lipids, glycolipids and phospholipids.

Phospholipids were isolated, extracted and separated by standard procedures prior to derivatization to their component fatty acid methylesters by sodium methoxide. Fatty acid methylesters were rapidly extracted into petroleum ether/diethyl ether and rapidly dried. They were then injected into a gas chromatograph (Agilent 5973 inert GC/MS system®, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (Capillary column, AT Silar length 30 m, film thickness 0.25 μm; carrier gas, helium; injector temperature, 250°C; detector temperature, 275°C). The oven temperature was controlled at 50°C for 2 min and then increased at a rate of 10°C min^{-1} to 200°C and maintained there for 20 min. A standard mixture containing all fatty acid methylesters (Sigma-Aldrich, MO, USA) was injected for calibration each day.

To evaluate the percentage of erythrocyte haemolysis with the osmotic variation of external mean (normal value in hyperosmotic solution of >70%; normal value in hyposmotic solution of <30%), we used an Osmored Monotest kit (glycerol 1.3%). The reticulocyte absolute number was assessed with a Sysmex XE-2100 Automated Hematology System by fluorescent flow cytometry.

The serum samples for the determination of erythropoietin were frozen within 1 h of their collection at a temperature of −40°C. All samples were analysed at the same time after the study. Erythropoietin was measured using a commercially available ELISA (normal values 4.3–32.9 units/l; Sangui BioTech, Inc., Santa Ana, CA, USA).

Lipid peroxidation was quantified by measuring concentrations of malonyldialdehyde, by spectrophotometry at 535 nm [16]. Malonyldialdehyde levels were expressed as nanomoles of thiobarbituric acid reactive substances formed per ml of plasma.

For antioxidant enzyme analysis, the haemolysate was prepared by 3-fold freezing and thawing the washed erythrocytes suspended in bi-distilled water. SOD activity was measured according to the Sun *et al.* [17] method, which consists of the inhibition of nitroblue tetrazolium reduction, with the use of xanthine–xanthine oxidase

as a superoxide generator. One unit of SOD activity is defined as the amount of protein that inhibits the rate of nitroblue tetrazolium reduction by 50%. Enzyme activity was expressed as units per mg of Hb. The principle of GPx activity assay was based on the decrease in the absorbance of reduced-nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm. GPx oxidizes reduced- to oxidized-glutathione, which is then reduced by GR (Fig. 1). In the last reaction, while NADPH is oxidized to $NADP^+$, the absorbance of NADPH at 340 nm begins to decrease steadily. GPx activity of erythrocytes was calculated per gram of Hb by measuring the absorbance change per minute and by using the molar extinction coefficient of NADPH [18]. The activity of GR was derived from oxidation of NADPH and was calculated per gram of Hb [18].

Statistical analysis

Descriptive statistics are presented as median and inter-quartile range (IQR). Due to the non-normal distribution of some variables and the limited sample size, non-parametric tests were used to assess differences after treatment and included the Wilcoxon rank-sum test for paired comparisons and the Mann–Whitney *U*-test for unpaired comparisons, the latter calculated on 6 months after treatment percent changes from the baseline value (SPSS version 15.0; SPSS, Inc., Chicago, IL, USA). To investigate dependence among the study variables, Spearman's correlation analysis was performed in the LVRS group by using the means of percent changes in the main respiratory parameters, erythrocyte osmotic resistance, reticulocyte absolute number, erythropoietin serum level and variation of SOD, GPx and GR levels, with data expressed as ρ coefficient and *P*-values. A *P*-value of <0.05 was considered significant.

RESULTS

Baseline inter-group analysis

Data at the baseline are summarized in Table 1. No significant difference in age, smoking history, disease severity or medication use (data not showed) was found between the two study groups.

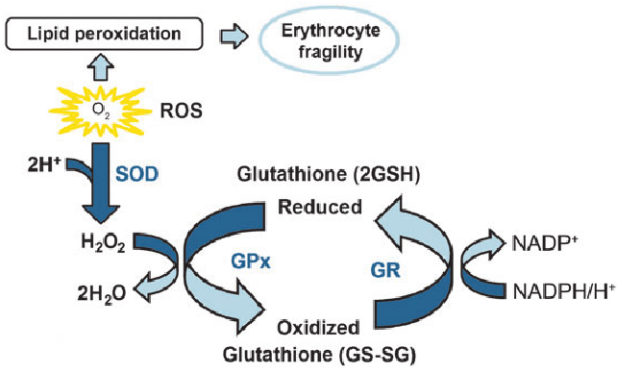

Figure 1: The antioxidant enzyme cycle and ROS inactivation. SOD catalyses the dismutation of superoxide radical (O_2) to hydrogen peroxide (H_2O_2). GPx neutralizes hydrogen peroxide by taking hydrogens from two reduced-glutathione (GSH) molecules resulting in two H_2O and one oxidized-glutathione (GS–SG) complex. Then, GR regenerates GSH from GS–G with the formation of nicotinamide adenine dinucleotide phosphate ($NADP^+$).

The comparison with normal reference values revealed an increment of erythrocyte haemolysis in hyperosmolar and hyposmolar solutions, reticulocytes absolute and erythropoietin levels. The analysis of fatty acid composition demonstrated a decrement of all membrane unsaturated fatty acids, with a lower unsaturation index and a higher malonyldialdehyde level. On the contrary, levels of erythrocyte antioxidant enzymes were lower than normal values.

Post-treatment intra-group analysis

Post-treatment data are shown in Table 2. In the LVRS group, all patients were available for the 6-month follow-up. In the RR group, 1 patient died, and the cause of death (car accident) was apparently unrelated to the lung disease. In both groups, all patients continued receiving combined inhaled therapy, with none of them requiring oral steroid therapy.

After LVRS, significant improvements were observed in most of the respiratory and symptomatic variables. The mean daily dosage of the combined inhaled therapy also significantly decreased in LVRS patients: beclomethasone [from 1.2 (IQR 1.0–1.5) to 0.7 (IQR 0.4–1.0) mg/day; $P = 0.001$] or budesonide [from 630.3 (IQR 710.5–550.2) to 410.4 (IQR 300.0–500.6) mg/day; $P = 0.001$] and salbutamol [from 360.4 (IQR 310.0–405.3) to 190.2 (IQR 150.4–250.6) mg/day; $P = 0.001$] or formeterol [from 38.1 (IQR 27.9–45.7) to 22.4 (IQR 10.0–35.6) mg/day; $P = 0.001$]. Unsaturated fatty acid content of the membrane improved with increment of unsaturated fatty acid total percentage (+10.0%, $P = 0.035$), unsaturation index (+17.5%; $P = 0.030$) and reduction in malonyldialdehyde concentration (−16.5%; $P = 0.020$). Structural erythrocyte membrane parameters improved: hyperosmolar (−21.0%; $P = 0.001$) and hyposmolar resistance (−18.0%; $P = 0.007$). Accordingly, percentage (−30.2%; $P = 0.002$) and absolute number (−15.5%; $P = 0.030$) of reticulocytes, as well as serum levels of erythropoietin (−16.7%; $P = 0.005$), decreased. Erythrocyte antioxidant enzymes also showed a significant increment: SOD (+60.0%; $P = 0.001$), GPx (+39.0%; $P = 0.006$) and GR (+24.5%; $P = 0.008$).

On the other hand, after 6 months from treatment, in the RR patients, we did not observe significant variations of any of these parameters. Combined inhaled therapy remained substantially unchanged.

Post-treatment inter-group analysis

Table 2 summarizes the comparison between the two groups 6 months after treatment. LVRS patients showed a greater amelioration in respiratory and functional parameters compared with RR. In the LVRS group, we also documented a significant improvement in unsaturation index, malonyldialdehyde concentration and erythrocyte osmotic resistance in hypo- and hyperosmotic solutions. Accordingly, we experienced a reduction in reticulocytes absolute number with a concomitant decrement of serum levels of erythropoietin, and an increment of antioxidant enzymes activities.

Correlation analysis

Correlation analysis is shown in Table 3. In the LVRS group, the reduction in lung hyperinflation and namely the decrease in residual

Table 1: Baseline median values with inter-group comparisons

Variables	LVRS group (N = 58) (median IQR)	RR group (N = 56) (median IQR)	LVRS vs RR P-value
Age, year	60.8 (52.8–69.7)	60.1 (51.5–70.5)	0.134
Smoking history, pack-year	31.0 (22.2–41.4)	33.3 (22.3–43.5)	0.427
FEV_1, % predicted	33.3 (27.1–40.2)	34.6 (26.6–40.1)	0.095
RV, % predicted	195.5 (164.0–221.8)	190.1 (162.1–231.5)	0.122
DLCO, mmol/kPa/min	3.8 (3.0–4.5)	3.9 (3.3–4.6)	0.198
paO_2, mmHg	65.0 (60.3–70.2)	63.6 (58.4–67.8)	0.314
$paCO_2$, mmHg	47.1 (40.1–52.2)	44.3 (42.3–52.8)	0.083
6-minute walking test, m	370.1 (342.3–410.1)	375.8 (330.1–430.9)	0.076
MRC dyspnoea index	3.0 (2.0–4.0)	3.1 (2.0–4.0)	0.144
SGRQ (100–0%)	27.3 (12.1–39.1)	25.7 (10.5–42.3)	0.323
Haemoglobin, g/dl	15.0 (14.5–15.4)	15.1 (14.5–15.5)	0.234
Hyperosmolar resistance, (% haemolysis)	84.3 (79.0–87.1)	85.5 (80.3–88.4)	0.355
Hyposmolar resistance, (% haemolysis)	33.3 (30.2–35.6)	32.6 (30.5–34.3)	0.217
Reticulocyte, absolute number (10^6 µl)	0.08 (0.05–1.10)	0.07 (0.03–1.13)	0.068
Reticulocyte, (%)	2.8 (2.5–3.1)	2.7 (2.5–3.0)	0.226
Erythropoietin (U/l)	40.3 (35.6–45.4)	39.7 (34.5–46.2)	0.075
Saturated fatty acids (%)	54.3 (49.1–59.4)	54.5 (50.2–59.0)	0.818
16 : 0 palmitic acid	34.9 (30.0–38.8)	35.0 (30.2–39.0)	0.923
18 : 0 stearic acid	15.7 (13.3–17.4)	15.0 (13.1–16.8)	0.079
20 : 0 arachidic acid	1.3 (0.5–19.1)	1.2 (0.5–19.4)	0.713
Unsaturated fatty acids (%)	45.7 (40.1–51.0)	45.5 (39.8–51.5)	0.871
18 : 1 oleic acid	20.0 (18.5–23.0)	21.0 (19.5–23.5)	0.085
18 : 2 linoleic acid	8.8 (8.0–9.5)	8.6 (8.0–9.9)	0.213
20 : 4 arachidonic acid	10.2 (8.0–12.0)	10.5 (8.5–12.5)	0.871
Saturated/unsaturated fatty acid	1.2 (0.9–15.2)	1.2 (0.9–15.4)	0.892
Unsaturation index[a]	98.0 (90.5–102.3)	96.2 (89.9–104.5)	0.542
Malonyldialdehyde (nmol/ml)	2.3 (2.2–2.4)	2.4 (2.2–2.5)	0.341
Superoxide dismutase (U/mgHb)	10.2 (9.0–11.4)	10.5 (9.2–12.0)	0.569
Glutathione peroxidase (U/gHb)	20.7 (19.5–21.9)	21.0 (20.0–22.0)	0.127
Glutathione reductase (U/gHb)	3.1 (2.1–4.2)	3.4 (2.4–4.7)	0.096

LVRS: lung volume reduction surgery; RR: respiratory rehabilitation; IQR: inter-quartile range; FEV_1: forced expiratory volume in 1 s; RV: residual volume; DLCO: diffusion lung carbon monoxide; MRC: medical research council; SGRQ: St. George's respiratory questionnaire.
[a]Sum of percentage by the weight of each unsaturated fatty acid times the number of unsaturated bonds.

volume (RV) was significantly correlated with the amelioration of hyper- (P = 0.019) and hyposmotic (P = 0.006) resistances. Significant direct correlations were found between RV and the absolute number of reticulocytes (P = 0.037) and erythropoietin (P = 0.040). RV improvement was also inversely correlated with the changes in the concentration of SOD, evidencing the relationship between lung hyperinflation and oxidative stress. This correlation was less evident for GPx and GR. This finding was likely due to the dependence of these two enzymes on the reduced-glutathione (Fig. 1), whose increment is probably slower than that of SOD.

DISCUSSION

An abnormal and prolonged inflammatory response to chronic exogenous 'noxae' with an increment of inflammatory mediators [5], proteases [11] and ROS (i.e. hydrogen peroxide, superoxide anion, hydroxyl radical, organic hydroperoxide) is a common finding during emphysema. All these events, detectable in pulmonary tissue, peripheral blood and partly in lung fluids, slowly cause destruction of alveolar wall epithelial cells, which represents the peculiar anatomical picture of the disease. Higher circulating ROS levels can induce variation of cell wall lipid composition, with lipid peroxidation of the normal poly-unsaturated fatty acids [6, 7, 19–22]. The lack of a proper synthesis of antioxidant enzymes able to contrast the damages of ROS overproduction and the constant trauma received during the microcirculation makes the erythrocytes the ideal target to monitor the negative effect of oxidative stress. Cell membrane in erythrocyte regulates the cellular volume [23], which is critical in tolerating extreme deformation while passing through the spleen [24]. Erythrocyte fragility causes the intra-splenic haemolysis with a reduction in cell half-life, thus aggravating hypoxia and stimulating the increment in the absolute number of reticulocytes as well as a hypersecretion of erythropoietin [25].

Mechanisms of protection against ROS are physiologically represented by enzymatic antioxidant defenses. In emphysema disease, the chronic hyperproduction of ROS inevitably results in a progressive consumption of all these endogenous antioxidant enzymes [4]. All these data are confirmed in our study where emphysematous patients presented a significant reduction in erythrocyte content of all such enzymes compared with what is determined in non-emphysematous subjects.

Six months after treatment, only patients who underwent LVRS presented a significant amelioration of respiratory parameters as well as a common shift to normal unsaturated fatty acid content and erythrocyte osmotic resistance with a concomitant increment of antioxidant enzymes. On the other hand, these changes were not detected in patients who had only RR, in which most of the altered parameters still persisted.

Table 2: Six-month post-treatment median percentage changes with intra- and inter-group comparisons

Variables	LVRS Changes %	RR Changes %	LVRS vs RR *P*-value
FEV_1, % predicted	+26.0***	+6.5*	0.019
RV, % predicted	−20.5***	+0.2	<0.001
DLCO, mmol/kPa/min	+4.0	+2.5	0.060
paO_2, mmHg	+7.5*	+4.0	0.045
$paCO_2$, mmHg	−3.0*	−3.0	0.030
6-minute walking test, m	+12.5*	+5.5*	0.024
MRC dyspnoea index	−60.0***	−33.3**	0.005
SGRQ (100–0%)	−23.1**	−8.0*	0.015
Haemoglobin (g/dl)	−14.0*	+0.9	0.008
Hyperosmolar resistance (% haemolysis)	−21.0***	+3.0	0.001
Hyposmolar resistance (% haemolysis)	−18.0***	−2.5	0.015
Reticulocyte, absolute number (10^6 μl)	−15.5**	+12.5*	0.005
Reticulocyte, %	−30.2**	+10.5*	<0.001
Erythropoietin (U/l)	−16.7**	−5.0	0.013
Saturated fatty acids (%)	−10.0*	+3.5	0.003
16 : 0 palmitic acid	−11.0*	+4.0	0.060
18 : 0 stearic acid	−3.0	+25.0**	0.070
20 : 0 arachidic acid	−66.7***	+6.0	<0.001
Unsaturated fatty acids (%)	+12.0**	−4.0	0.014
18 : 1 oleic acid	+1.0	+0.8	0.120
18 : 2 linoleic acid	+11.0*	−0.5	0.010
20 : 4 arachidonic acid	+24.0**	−6.6	<0.001
Saturated/unsaturated fatty acid	−26.1**	+8.0*	<0.001
Unsaturation index[a]	+17.5*	−7.0	0.009
Malonyldialdehyde (nmol/ml)	−16.5*	+2.0	0.014
Superoxide dismutase (U/mgHb)	+60.0***	−3.5	0.002
Glutathione peroxidase (U/gHb)	+39.0**	+2.0	0.008
Glutathione reductase (U/gHb)	+24.5**	+7.0	0.038

LVRS: lung volume reduction surgery; RR: respiratory rehabilitation; IQR: inter-quartile range; FEV_1: forced expiratory volume in 1 s; RV: residual volume; DLCO: diffusion lung carbon monoxide; MRC: medical research council; SGRQ: St. George's respiratory questionnaire.
[a]Sum of percentage by the weight of each unsaturated fatty acid times the number of unsaturated bonds.
Intra-group *P*-value *< 0.05, **< 0.01, ***< 0.001.

We have previously demonstrated that operated emphysematous patients showed a decrement of cytokines with a reduction in the pulmonary inflammatory response [11]. These subjects probably achieved an attenuation of the oxidative stress with a reduction in ROS production. The reduction in oxidative stress within the erythrocytes is tested by the normalization of antioxidant enzymes, with the restoration of the normal composition of unsaturated membrane fatty acids and the osmotic resistance recovery.

Our data evidenced in the LVRS group a maximal correlation between the reduction in lung hyperinflation, namely RV, which is the most sensitive parameter for LVRS benefits, and the amelioration of the erythrocyte osmotic resistance as well as the normalization of antioxidant enzymes. LVRS decreases thoracic hyperinflation and breathing workload, allowing re-expansion of compressed lung tissue and airways. All these changes, together with the elimination of sites where inflammatory mediators are produced and anti-proteases are consumed, can induce a reduction in local and systemic effects of emphysema, with a decreasing overproduction of ROS. The increase in erythrocyte osmolar resistance results in a conservation of oxyemia levels with negative feed-back on erythropoietin production results in a reduced number of circulating reticulocytes.

On the other hand, these changes cannot be observed in patients undergoing RR, which continue to deteriorate. As a matter of fact, RR may improve respiratory mechanics and gas exchange, achieving a significant relief from dyspnoea with an increment in exercise capacity, but it is not able to modify pulmonary static volumes and lung hyperinflation. In addition, RR does not interfere with the systemic inflammation and ROS production, since no change in lung tissue composition can be directly made. Interestingly, we did not find a significant decrement of reticulocytes and erythropoietin levels in RR despite the significant improvements in oxygen levels. This could be a further evidence of the role of haemolysis in reticulocyte increment during emphysema.

The limitations of our study are its being a retrospective nonrandomized clinical trial as well as the relatively small study population and the marginal evaluation of the combined inhaled therapy. The bias is partially counterbalanced by the homogeneity of the two groups selected and treated by a single dedicated centre of care and investigation.

We can conclude that LVRS seems to significantly improve unsaturated fatty acid content and erythrocyte osmotic resistance over RR, also improving the levels of antioxidant enzymes. Correlations between these improved parameters and reduction in RV with normalization of erythropoietin levels and reticulocytes count suggest that the reduction in lung hyperinflation with the elimination of emphysematous inflammatory tissue potential site of ROS production may account for the clinical improvements after surgery.

The outcomes of this analysis indicate the relation between moderate-to-severe emphysema and circulating inflammatory

Table 3: Correlation analysis (Spearman's test) in the LVRS group

Variables	Changes% FEV_1	Changes% RV	Changes% DLCO
Changes %, hyperosmotic resistance			
ρ coefficient	0.246	−0.432	0.153
P-value	0.199	0.019	0.427
Changes %, hyposmotic resistance			
ρ coefficient	0.026	−0.497	0.208
P-value	0.892	0.006	0.278
Changes %, reticulocyte absolute number			
ρ coefficient	−0.153	0.389	−0.046
P-value	0.427	0.037	0.812
Changes %, erythropoietin			
ρ coefficient	−0.228	0.383	−0.285
P-value	0.235	0.040	0.134
Changes %, superoxide dismutase			
ρ coefficient	0.223	−0.731	0.198
P-value	0.233	<0.001	0.304
Changes %, glutathione peroxidase			
ρ coefficient	0.112	−0.363	0.149
P-value	0.565	0.053	0.440
Changes %, glutathione reductase			
ρ coefficient	0.079	−0.434	−0.326
P-value	0.683	0.019	0.084

FEV_1: forced expiratory volume in 1 s; RV: residual volume; DLCO: diffusion lung carbon monoxide.

mediators. Accordingly, LVRS might be considered not only a surgical technique with anatomical–functional respiratory effects, but also a biohumoral pulmonary and systemic strategy with a positive nutritional, metabolic and hormonal impact, significantly improving the clinical status of the patients and possibly delaying the natural history of the disease. Once again, we reaffirm the validity of this operation in selected patients despite the increasing criticism arising in the scientific world.

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