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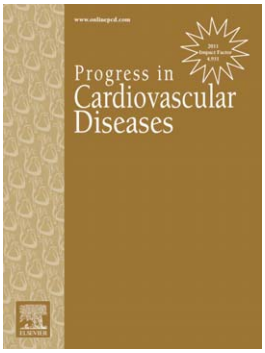

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Endothelial Dysfunction and Lung Capillary Injury in Cardiovascular Diseases

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Abbreviations

ACE- Angiotensin converting enzyme

AV – Alveolar volume

ATPase - Aadenosine triphosphatase

CAV – Caveolin

cGMP - cyclic guanosin monophosphate

CO2 – Carbon dioxide

DLco - Diffusing capacity for carbon monoxide

DLNO - Diffusing capacity for nitric oxide

EMT - epithelial-mesenchimal transition

ET1 - endothelin-1

GC - guanilate cyclase

HF – Hearrt failure

HFpEF – Heart failure-preserved ejection fraction

HFrEF – Heart failure-reduced ejection fraction

L-NMMA - NG-monomethyl-L-arginine

LA- Left atrial

LAP – Left atrial pressure

LV – Left ventricular

MCP-1 - Monocyte chemoattractant protein 1

mPAP - mean pulmonary artery pressure

NO – Nitric oxide

O2 - Oxygen

PH – Pulmonary hypertension

PDE5 - phosphodiesterase 5

Q – Pulmonary perfusion

sGC - soluble guanylate cyclase

TGF-α1 - Transforming growth factor

TNF-α - Tumor necrosis factor alpha

V/Q – Ventilation/perfusion

Abstract

Cardiac dysfunction of both systolic and diastolic origin leads to increased left atrial pressure, lung capillary injury and increased resistance to gas transfer. Acutely, pressure-induced trauma disrupts the endothelial and alveolar anatomical configuration and definitively causes an impairment of cellular pathways involved in fluid-flux regulation and gas exchange efficiency, a process well identified as stress failure of the alveolar-capillary membrane. In chronic heart failure (HF), additional stimuli other than pressure may trigger the true remodeling process of capillaries and small arteries characterized by endothelial dysfunction, proliferation of myofibroblasts, fibrosis and extracellular matrix deposition. In parallel there is a loss of alveolar gas diffusion properties due to the increased path from air to blood (thickening of extracellular matrix) and loss of fine molecular mechanism involved in fluid reabsorption and clearance. Deleterious changes in gas transfer not only reflect the underlying lung tissue damage but also portend independent prognostic information and may play a role in the pathogenesis of exercise limitations and ventilatory abnormalities observed in these patients. Few currently approved treatments for chronic HF have the potential to positively affect structural remodeling of the lung capillary network; angiotensin-converting enzyme inhibitors are one of the few currently established options. Recently, more attention has been paid to novel therapies specifically targeting the nitric oxide pathway as a suitable target to improve endothelial function and permeability as well as alveolar gas exchange properties.

Key Words: Endothelial dysfunction, lung capillaries, capillary unit.

Introduction

A typical feature of the failing left ventricle (LV) is the loss of its ability to relax and fill at normal pressures irrespective of the definition of heart failure (HF) based on preserved or reduced ejection fraction [1]. The pathophysiology of high end-diastolic pressures and its consequences on the lungs is complex and meaningful in clinical conditions involving both acute or chronic filling pressure elevation [2] . The most remarkable manifestation of an acute pressure challenge on lung capillaries is pulmonary edema, while pulmonary hypertension (PH) and right ventricular failure are the late consequences [3] .

Despite the fact that these unfavorable conditions are highly prevalent and portend a negative outcome [4, 5] the pathophysiology of left-sided heart disease associated with lung capillary injury is not completely understood and is infrequently considered as potential therapeutic target. [6] In this article we review the pathophysiological bases and clinical implications of lung capillary injury and its consequences in patients with left-sided heart disease.

Lung Capillary Injury: Pathophysiological Bases

There is strong preclinical evidence that lung microcirculation impairment warrants attention as a determinant of unfavorable clinical outcome [7]. When lung capillaries are exposed to an excessive hydrostatic pressure a stress failure phenomenon occurs, initially described by West and co-workers in a series of experimental preparations in different animal models [8]. Stress failure challenges the anatomical integrity of the alveolar-capillary unit, alters endothelial permeability, fluid filtration and reabsorption and definitively leads to gas exchange impairment. Interstitial edema or alveolar flooding are the most impressive consequences of stress failure [8]. Alternatively, when left atrial (LA) pressure elevation is less striking and is long-lasting, a true remodeling of capillaries and small arteries takes place. A cascade of hormonal and cytotoxic activation is involved in the remodeling process that unequivocally leads to abnormalities in gas exchange (Figure 1) [9].

Tsukimoto et al. [10] studied the sequential disruption of the capillary endothelial and alveolar epithelial layers during a stepwise increase in hydrostatic pressure, reproducing the transition from interstitial leakage of protein (low permeability stage) to alveolar lumen leakage of protein and erythrocytes (high-permeability stage of pulmonary edema). A number of animal studies that have focused on the biological features of alveolar stress failure have shown that mechanisms other than mechanical injury may contribute to capillary stress [11, 12]. Induction of volume overload through 0.5 ml/min^{-1}/kg^{-1} saline solution infusion for 180 min in the rabbit pulmonary artery was associated with a 44% portion of fluid accumulating in the interstitial space, ultrastructural changes, and impairment of gas transfer. [11] Development of hydraulic edema leads to activation of metalloproteinases, which degrades matrix proteo glycanstherevy altering the composition of the plasma membrane and contributing to increased endothelial membrane fluidity. The weakened tensile strength of the membrane potentiates endothelial stress failure [12]. These findings might explain the acute rise in pulmonary hydrostatic pressure and pulmonary edema seen in humans, even if the pathophysiological correlates of alveolar–capillary stress failure in patients with cardiac disease have not been extensively investigated. In a study of 53 patients with acute cardiogenic pulmonary edema, injury of the alveolar–capillary barrier was associated with increased levels of plasma pulmonary surfactant associated proteins A and B and tumor necrosis factor (TNF)- α [13]. Persistence of elevated levels of TNF-α after pulmonary edema resolution may reflect pulmonary inflammation and explains why fluid accumulation can persist despite resolution of hydrostatic stress failure.

When pressure injury is sustained a true remodeling process takes place that might not be reversible. Several experimental models of PH due to cardiac dysfunction have brought important insights into this area. In a descending coronary artery ligation post MI model, abnormalities in the lung microcirculation consisted of increased oxidative stress and diffuse inflammation [14] .

Pacing-induced cardiomyopathy has been shown to produce alveolar–capillary membrane thickening as a result of excessive deposition of type IV collagen (the main component of the membrane lamina densa) [15]. Similar findings were reported in a guinea pig model where an increased lamina was not accompanied by an increased lung water content [16], suggesting that chronic proliferation rather than fluid accumulation predominates. In a mouse model of PH-HF-preserved ejection fraction (HFpEF) with LV hypertrophy, the LA pressure rise induced by aortic banding promoted impressive arteriolar remodeling, increased vascular oxidative stress, leucocyte infiltration and lung fibrosis after 4 weeks [17]. In addition, lung weight changes were due to tissue and vascular changes rather than extravascular lung water [17]. These features are reminiscent of the extracellular matrix thickening reported in patients with mitral stenosis and pulmonary venous pressure elevation [18, 19] in whom it accounts for the structural changes observed and might be viewed a safety mechanism against excessive fluid leakage from the capillaries. An increase in collagen content not observed in pre-capillary PH but typical of post-capillary PH is mediated by proliferation of

myofibroblasts termed “interstitial contractile cells (MYFs) [20]. Growth factors that can trigger proliferation are classical local growth factors, such as angiotensin II, endothelin 1 (ET-1), TNF-α and especially transforming growth factor (TGF)-α1 which has been shown to be the major inducer of epithelial-mesenchimal transition (EMT) in the fibrotic lung [21]. The caveolin family of proteins (Cav-1, Cav-2, Cav-3), which are the main structural component of caveolar membranes surrounding the vescicular invaginations arising from plasma membranes, is seemingly involved in the remodelling process through hyperactivation of the Janus Kinase/signal transducer and activation of the transcription (JAK/STAT) signaling cascade [22]. In mice knockout for Cav-2 there is a significant thickening of the alveolar septa and in the post- myocardial infarction (MI) model, Cav-1 and Cav-2 expression is reduced to undetectable levels [23].

Recently, Park et al. [7] found that lung microvascular endothelial cells exposed to cyclic mechanical strain in vitro released proinflammatory and profibrotic mediators identifying a specific putative role for the monocyte chemoattractant protein 1 (MCP-1).

The increase in lung interstitial connective tissue associated with chronic capillary hydrostatic overload results in increased extravascular fluid storage owing to increased production of an extracellular matrix component (mainly glycosaminoglycans) that has the potential to absorb and accommodate fluid in the interstitium. At least in cases of a subcritical rise in persistent LA pressure, this compensatory mechanism could prove beneficial by constraining fluid in the perivascular space without limiting gas diffusion [24].

Alveolar hypoxia is another potential mediator that may substantially affect the composition of the extracellular matrix by increasing the expression of genes that encode extracellular matrix proteins [25]. These structural modifications generally increase the impedance to gas transfer [26]. In patients with HF, assessment of lung diffusion capacity by measuring the alveolar membrane conductance component enables quantification of the anatomical and functional integrity of the alveolar–capillary unit, which provides prognostic insights [27]. Transition from stress failure to remodeling is a key step in the development of PH, and the true reversibility of this process is unknown.

Endothelial Dysfunction- Once arteriolar remodeling has developed, the endothelium plays an integral role in mediating the functional alterations of the pulmonary vasculature [28]. In the pulmonary circulation, the endothelium-mediated local control of vasomotility is primarily based on a balanced release of nitric oxide (NO) and ET-1 with the evidence suggesting that raised pulmonary pressure owing to left-sided heart disease is critically sensitive to an imbalance of these two opposing systems [29, 30].

Studies performed in rats with PH and documented capillary remodeling have provided insights into the role endothelial NO-mediated dysfunction attributable to impaired $Ca2^{+}$(i) endothelial homeostasis, as reflected by the lack of $Ca2^{+}$ (i) oscillations and attenuation of the response to mechanical stress or chemical stress with histamine, acetylcholine or thapsigargin (**FIGURE 2**) [31].

Studies with blockade of NO synthesis have provided evidence that endothelium-derived NO is a basic determinant of baseline pulmonary vascular tone, and a mediator of the dilating response to endothelium activation [32]. In normals, systemic infusion of NG-monomethyl-L-arginine (L-NMMA), an analog of L-arginine that inhibits NO synthase, raises pulmonary artery pressure, enhances hypoxia-induced pulmonary vasoconstriction, [33] and inhibits the lung diffusion of carbon monoxide by lowering alveolar–capillary membrane conductance [34]. In patients with HF, infusion of L-NMMA in the pulmonary circuit causes a dose-dependent vasoconstriction, which is partially attenuated by acetylcholine [28]. Human and animal studies suggest that NO-mediated pulmonary vasodilatation is impaired in left-sided heart disease. Porter et al. [35]assessed pulmonary artery diameter with intravascular ultrasonography and reported vessel

dilatation when acetylcholine was infused in patients with LV dysfunction and normal pulmonary artery pressure, but dilatation was refractory when the baseline pressure was elevated. Data that supports attenuation or loss of NO-dependent vasodilatation as a basic contributor to pressure elevation have also been provided by recording pulmonary blood flow velocity during intrapulmonary infusion of L-NMMA [29]. In healthy individuals and in patients with LV dysfunction with normal pulmonary vascular resistance, L-NMMA elicited a conspicuous vasoconstrictor response. This effect was attenuated when L-NMMA was infused in patients with HF and PH. Notably, vasoconstriction was similar in the three groups in response to phenylephrine.

Molecular Abnormalities in Alveolar Fluid Clearance-Fluid clearance from the alveoli to the capillaries is a process of vital importance. Na^+ transport across the alveolar epithelium helps to reabsorb fetal fluid [36], ensuring a proper thinness of the adult alveolar fluid, the so-called film, and keeping alveolar space free of fluid, especially in pathologic states when alveolar permeability [37] to plasma proteins has been increased. The alveolar type II cell transport of Na+ (**Figure 3**) provides the major driving force for water removal from the alveolar space. After uptake, Na^+ is pumped actively into the lung interstitium by Na^+-K^+ adenosine triphosphatase (ATPase). For an optimal gas exchange, the fine mechanisms that control alveolar Na+ and water metabolism are basically involved. Although disorders in lung diffusion in cardiac patients generally have been referred to as alterations of the endothelial and alveolar epithelial cells [38], experimental observations are also consistent with an involvement of alveolar water metabolism.

Interestingly, in rats overexpressing, by adenovirus gene transfer, the Na^+-K^+ ATPase β_1-subunit, there is an increase in liquid fluid clearance. In the same model, Na^+ transport and alveolar fluid clearance in the presence of elevated left atrial pressure (LAP) was not different from that in rats studied at normal LAP [37]. Hypoxia, another common consequence of chronic HF, is also capable of inhibiting alveolar Na^+-K^+ ATPase function and transalveolar fluid transport [39]. These findings support the intriguing hypothesis that impaired Na^+-K^+ ATPase gene expression occurs during acute lung injury, and provide evidence that the result of a pressure and/or a volume overload on the lung circulation is an increase in capillary permeability to water and ions and disruption of local mechanisms for gas exchange.

Detection of Lung Capillary Injury in Clinical Practice: Relevance of Gas Diffusion Abnormalities

At variance with the systemic circulation, studies directly measuring endothelial function in the lung are limited to the invasive approach or to a simple, and in part questionable, correlation with a systemic endothelial flow-mediated response [40]. Nonetheless, there is a strong rationale for taking the gas diffusing properties across the alveoli as an indirect indicator of endothelial permeability and therefore endothelial activity [26] .

Measurement of lung diffusing capacity for carbon monoxide (DLco) or nitric oxide (DLNO) is generally used in clinical practice to evaluate the effectiveness of diffusive oxygen (O2) transport [41] . As originally suggested by Roughton and Forster [42], for a given alveolar volume and hemoglobin concentration, gas diffusion depends on two resistances arranged in series according to the following equation:

$1/DLCO = 1/Dm + 1/ \theta CO \times Vc$

Where Dm is the alveolar-capillary membrane conductance, ɵCO is the rate of CO uptake by the whole blood in combination with hemoglobin measured in vitro and Vc is the capillary blood volume. Changes in Dm track structural alterations of the alveolar-capillary barrier, providing a sensitive noninvasive indication of microvascular integrity in health and disease. Vc is related to pulmonary capillary wedge pressure. In stable HF patients, Vc tends to increase in order to compensate for low Dm patients and this correlates with unfavorable hemodynamics and increased pulmonary vascular resistance [43]. Reduction in the Dm component rather than changes in Vc accounted for observed gas diffusing abnormalities. Several reports [43-48]43-48 have confirmed and expanded these findings and in studies in which alveolar volume (AV) has been measured, abnormalities in DLco persisted even after AV normalization. Patients with HF and diabetes comorbidity exhibit a more severe Dm impairment compared to patients with similar hemodynamic dysfunction without diabetes [48].

Pathophysiologic Correlates. Studies investigating the pathophysiologic role of a reduced Dm in HF patients have addressed the question as to whether Dm changes are fixed or there is also a variable component related to interstitial edema and loss of fluid permeability. According to the basic experimental evidence that pulmonary interstitial fluid accumulation is secondary to a dysregulation of Na+ handling, changes in Dm following saline infusion have been investigated in patients with chronic HF of varying severity. In a study performed in post-MI patients with normal LV systolic function, an infusion of ~800 mL of saline reduced Dm by 13% [49]. In patients with mild to severe HF, a 150 ml infusion of saline produced a significant Dm reduction equivalent to 750 ml saline, whereas a 750 mL infusion of isotonic glucose solution did not decrease DLco and Dm [50]. None of these infusions caused changes in right atrial or pulmonary wedge pressures. These findings support the idea that part of Dm abnormalities are fluid-dependent and that Na+ infusion may be, even in a small amount, a challenge for cellular pathways involved in alveolar fluid clearance and capillary fluid reabsorption.

Pulmonary abnormalities, and specifically those related to gas diffusion capacity, can explain part of the symptoms and functional limitations encountered in HF. Dm at rest and relative changes on exertion correlate with O2 uptake at peak exercise [51]. The correlation, however, is even greater between Dm and the excessive ventilatory requirement to carbon dioxide (CO2) output, which is typical in these patients [52].

The putative role of lung diffusion abnormalities in the pathophysiology of exercise limitation in HF has not been definitively appreciated because of the lack of significant O2 desaturation during exercise. In HF patients, however, despite the fact that pulmonary perfusion (Q) may be significantly reduced, the ability to appropriately recruit Dm for a given Q preserves the Dm/Q ratio and prevents O2 from significant drops [33]. There is, however, documentation that development of subclinical pulmonary edema during exercise is a common finding in HF patients as suggested by a significant and persistent Dm and Dm/Vc reduction during the recovery phase of exercise [53]. This may result in an increased sensation of dyspnea by activation of J receptor afferents, and there is a suggestion that the DM/Q ratio decreases and significant hypoxemia on exercise develops in some post-transplant patients. This is explained by the fact that pulmonary lung perfusion is reversed to normal in the presence of fixed ultrastructural membrane changes leading to exercise ventilation/perfusion (V/Q) mismatching.

Therapeutic Perspectives

In contrast with the increasing evidence of a pathophysiologic role, the opportunity to consider lung capillary function and alveolar fluid reabsorption as therapeutic targets are still underestimated [54]. The importance of considering altered gas exchange as a target of treatment is underscored by the demonstration that despite major airway abnormalities, cardiac patients may improve with tailored

therapy; DLco remains low up to several years after heart transplantation and a relationship has been demonstrated between the time course of the disease and impedance to gas exchange [55]. These findings are confirmatory of a progressive lung capillary remodeling process and suggest that a reduction in DLco reflects, at least in part, fixed structural changes. Consistently, in a prospective survival study where Dm and Vc were investigated, Dm was the only independent pulmonary predictor of worse prognosis in HF patients [56].

Despite the lack of evidence of complete DLco and Dm normalization with treatment, a favorable modulatory activity on these variables by angiotensin converting enzyme (ACE)-inhibitors has been repeatedly reported [57]. This effect appears promptly after drug administration, which persists over time and is unrelated to a lowering of pulmonary pressure. These mechanisms seem also to be involved in the overall benefits on survival produced by this class of drugs [56]. Factors that reasonably may underlie the improvement in gas exchange with ACE-inhibitors include a modulation in extracellular matrix synthesis and collagen turnover, or an improvement in endothelial permeability and increased alveolar epithelial reabsorption of Na+ and fluid [58]. Interestingly, an association has been found between DLco and ACE genotype polymorphism [47] , implying that higher ACE-inhibitors doses are very likely required for treating lung abnormalities of HF patients with ACE DD-genotype. Beta-blockers do not provide a similar reverse remodeling of the alveolar pneumocytes such as that occurring in the biological properties of cardiomyocites. A 6 month follow-up with carvedilol did not promote any improvement in DLco or Dm [59].

The concern that antiarrhythmic treatment with amiodarone may exert untoward effects on lung diffusion may very likely be excluded by the demonstration in a relatively large population of HF patients that no DLco changes occurred after 1 year of amiodarone treatment [60]. Since DLco may not adequately reflect abnormalities intrinsic to the alveolar epithelial and capillary layer, this question awaits further and more detailed studies.

NO Pathway Overexpression. Since NO release by the pulmonary and venous endothelium is crucial for normal lung physiology, i.e. vascular permeability and molecular O2 transfer from the alveolus to its uptake by hemoglobin [33], its modulation/overexpression is seen as a meaningful target. In this regard, the most recent attention has been toward targeting the NO pathway at different downstream levels through overexpression of the cyclic guanosin monophosphate (cGMP) by two mainstay compounds, the phosphodiesterase 5 (PDE5) inhibitors and guanilate cyclase (GC) activators/stimulators. PDE-5 is the predominant isoenzyme that metabolizes cGMP, the second messenger of NO, which is highly generated in the smooth muscle cells of pulmonary arteries and veins. PDE-5 activity is increased in various experimental models of PH [61]. In remarkable experiments by Yin et al, sildenafil given to a rat model of PH secondary to massive left ventricular hypertrophy was capable of reversing endothelial dysfunction and alveolar capillary remodeling, including capillary and small artery thickness that reversed their structural changes to normal after active drug therapy [62].

In humans, inhibition of PDE-5 with sildenafil restores a normal cGMP transpulmonary gradient (increased arteriolar and capillary release) in patients with HF and high pulmonary vascular resistance [63].

Evidence of the role of PDE5 inhibition on the pulmonary vasculature is increasing. Findings by our group [64] suggest a long-term (1 year) sustained role for sildenafil in reducing pulmonary pressures and especially improving gas exchange (DLCO, Dm and Dm/VA) in a subset of patients with moderate to severe PH and HFpEF. In the placebo arm, there was a progressive increase in mean pulmonary artery pressure (mPAP). The associated rise of pulmonary arteriolar resistance with stable pulmonary arterial wedge pressure

suggests that changes in mPAP in these patients is due to evolution of the vascular disease rather than LV diastolic dysfunction.

Recently, the introduction of additional compounds that target the downstream intracellular NO pathway by activation/stimulation of soluble guanylate cyclase (sGC) has offered another opportunity to overexpress cGMP signaling. Specifically, two trials [64, 65] have tested the acute effects of riociguat, a first in class compound that shows a dual mode of action: it sensitizes sGC to endogenous NO and directly stimulates sGC independently of NO.

Both the LEPTH [patients with HF-reduced ejection fraction (HFrEF)] [65] and the DILATE (patients with HFpEF) [66] trials have investigated hemodynamic effects, showing a good safety and tolerability profile, even with riociguat at different doses. However, no data has thus far been reported on endothelial function and gas exchange.

Finally, there is the interesting perspective that interventions aimed at improving systemic endothelial function, such as aerobic exercise training, may also favorably impact gas exchange and pulmonary capillary properties. In a stable HF cohort, exercise training has been shown to improve gas diffusion through a combined effect on Dm and Vc and the normalization of alveolar volume. This suggests that repeated episodes of increased blood flow (i.e., shear stress) with exercise or metabolic effects of training may be the basis for a chronic stimulus for the release of endothelial paracrine agents, such as NO, that control vascular tone and permeability. These effects may not be confined to the exercising limbs but would be imposed throughout the vasculature, including the lungs [67]. Information is still limited but represents a promising area for further investigation.

Conclusions

Development of cardiac dysfunction exposes the lung microcirculation to a pressure and/or volume overload; functional, physiologic and anatomical consequences have been outlined in different animal models and experimental conditions. Acutely, mechanical injury to the capillaries leads to the so-called stress failure, a process that causes endothelial and alveolar cell breakdown and impairs cellular pathways involved in control of vascular tone, fluid permeability and reabsorption. When the lung microcirculation is chronically challenged a typical remodeling process takes place characterized by fibroblast proliferation, fixed structural membrane alterations (i.e., extracellular matrix collagen proliferation) and re-expression of fetal genes. Remodeling leads to a sustained reduction in gas diffusion that does not appear to be totally reversible, as evidenced by the fact that abnormalities in alveolar gas diffusion persist for several years following heart transplantation. Some therapeutic approaches that favorably impact the natural course of cardiac remodeling, such as ACE-inhibitors, exert a positive effect on alveolar properties and gas exchange. Therapies aimed at increasing lung capillary NO availability, such as exercise training, seem to be a promising opportunity for reversing capillary dysfunction and diminished alveolar membrane diffusive properties encountered in patients with HF.

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Legend For Figures

Figure 1 *Elevation in left atrial pressure and proposed sequence of events that lead to capillary stress failure and remodelling of the capillaries and alveolar surface .*

An abnormal increase in left atrial pressure leads to alveolar stress failure, a process that disrupts the anatomical and functional properties of the capillaries (loss in permeability and fluid leakage) and alveoli unit (loss of absorption) but is reversible. In chronic PH, a superimposition of additional factors other than mechanical stress, such as neurohumoral, cytotoxic, hypoxic, and genetic factors, injure lung capillaries and alveolar spaces further, which triggers a remodeling process that ultimately result in some degree of protection against edema formation but impairment in gas exchange physiology. Abbreviations: ET1, endothelin-1; PH, pulmonary hypertension; TNF-α, tumor necrosis factor-α, TGF-β= transforming growth factor β; Cav-1=caveolin 1, Cav-2= caveolin 2 (adapted from Guazzi M et al Nature Review Cardiol 2010) [9].

Figure 2. *Schematic presentation of the molecular and cellular pathways involved in alveolar fluid clearance.*

Na^+ enters the apical membrane of alveolar type II cells mainly through the amiloride-sensitive epithelial Na^+ channels, and is then transported across the basolateral membrane in to the interstitium through the ouabain-inhibitable Na^+/K^+ ATPase pump. On the apical surface of type II cells there is also a glucose cotransport system for Na^+. The passive Na^+ transport generates an osmotic gradient that induces removal of excessive intralveolar fluid. In several clinical conditions such as HF, a defect of this mechanism predisposes patients to pulmonary edema regardless of Starling forces and lymphatic drainage (adapted from Guazzi M J Card Fail 2008) [17].

Figure 3. *Experimental insights on the molecular mechanisms that lead to endothelial dysfunction once a capillary remodelling develops secondary to left sided PH.*

9 weeks of aortic banding determined a lack of endothelial NO release during acetylcholine stimuli assessed in vivo with luminescence technique. This was accompanied by a lack of physiological oscillations in Ca^{2+} endothelial handling working as a trigger for the occurrence of important cytoskeleton dysorganization (adapted from ref. Yin J Circ Heart Fail 2011;4:198)[61]

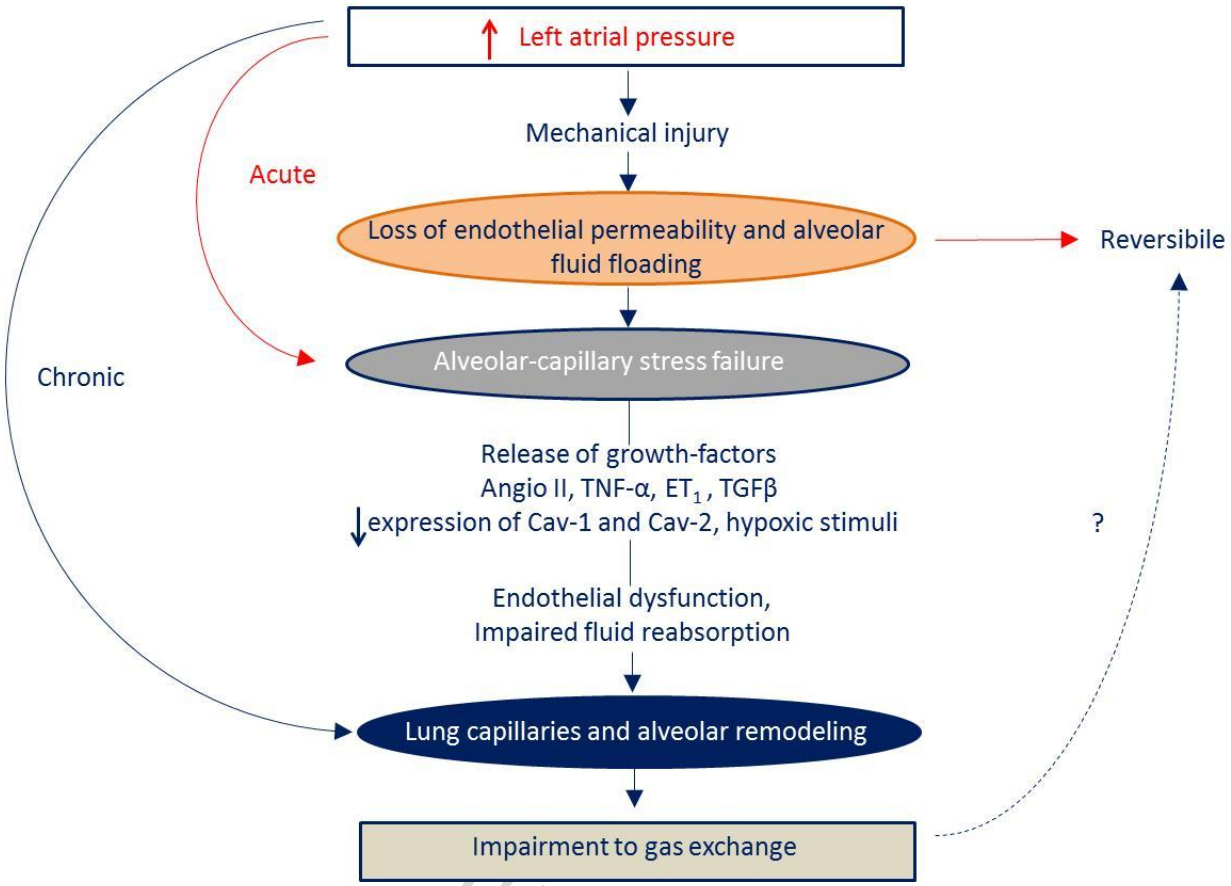


Fig. 1

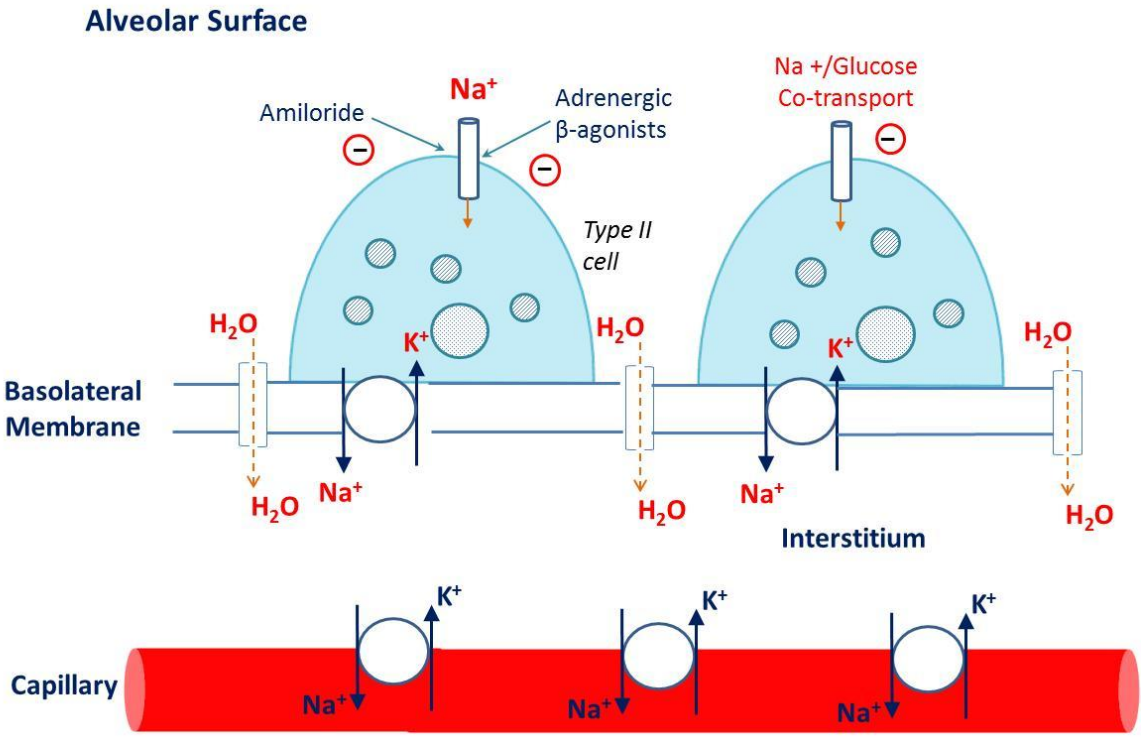
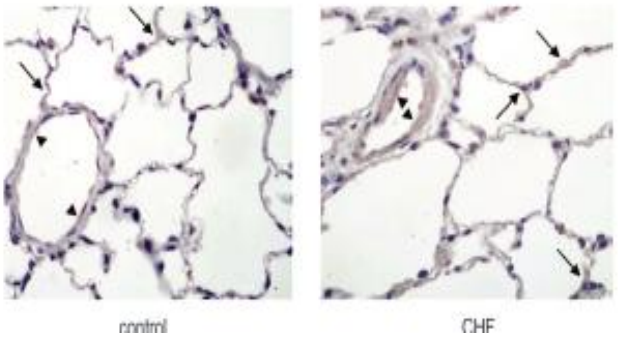
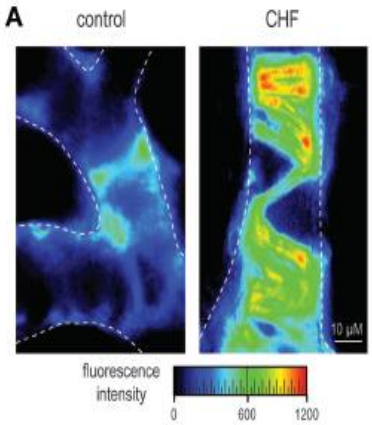


Fig. 2

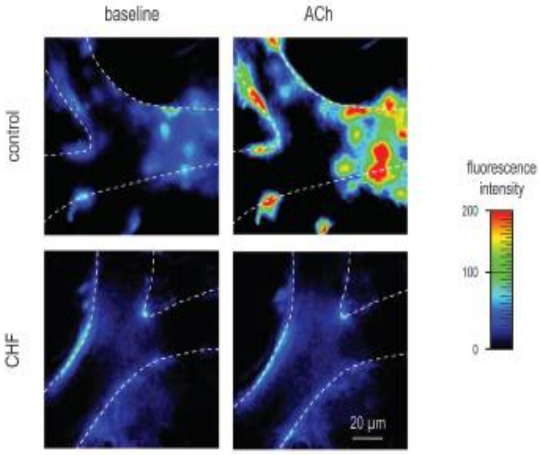
Alveolar-Capillary



Actin Cytoskeleton



Capillary NO Release to



Capillary $[Ca^{2+}]$ Oscillations

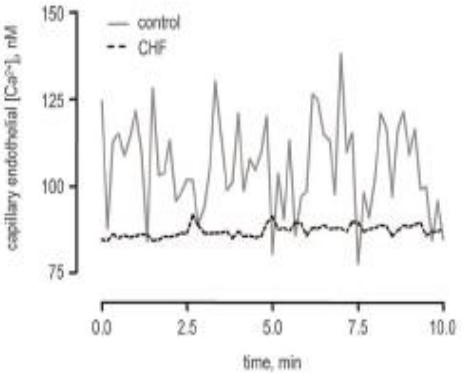


Fig. 3