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Role of BNP and echo measurement for pulmonary hypertension recognition in patients with interstitial lung disease: An algorithm application model

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KEYWORDS
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Summary
Background: This study evaluated the role of echocardiography and BNP in patients with interstitial lung disease (ILD), to identify those with PH and RV dysfunction. The aims of this study were: 1-to evaluate the accuracy of an algorithm including BNP, DLCO and echocardiographic measurements to identify PH and RV dysfunction; 2- to evaluate BNP and Echo values concordance in relation to right catheterization measurement.
Methods: We analyzed 113 patients with diagnosis of ILD. Echo examination included: Pulmonary systolic, diastolic and mean Arterial Pressure (PAPs, PAPd, PAP mean), End-Diastolic and End-Systolic right ventricle diameters, Inferior Caval Vein diameter, and Tricuspid Annular Plane Systolic Excursion (TAPSE). Patients revealing increased PAPs at echocardiography underwent to catheterization.
Results: Patients with PAPs $>$ 40 mm Hg (37 patients), PAPmean $\geq$ 25 mm Hg (23 patients) and PAPd $\geq$ 20 mm Hg showed BNP increased (157 $\pm$ 96 vs 16 $\pm$ 14 pg/ml $p = 0.004$; 201 $\pm$ 120 vs 28 $\pm$ 17 pg/mL; 124 $\pm$ 88 vs 23 $\pm$ 18 pg/ml $p < 0.001$) as patients with TAPSE $\leq$16 mm (25 patients) (145 $\pm$ 104 vs 26 $\pm$ 21 pg/ml $p < 0.001$). In catheterized patients (37 patients) BNP was increased in patients with invasive PAPs $>$ 40 mm Hg (165 $\pm$ 112 vs 29 $\pm$ 14 pg/ml $p < 0.02$), as well as in patients with Wedge pressure $>$ 14 mm Hg (199 + 153 vs 54 + 39 pg/mL; $p = 0.01$). ROC Curve analysis showed that elevated values of BNP, PAPs, PAP mean are able to assess PH.

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On the other hand, lower values of DLCO (<40%) and TAPSE (≤16 mm) detect PH. Logistic regression analysis of the previous parameters, confirmed their diagnostic role in PH detection.
Conclusions: In patients with ILD, an algorithm including BNP, DLCO and echocardiography could be useful for non invasive screening of PH.
Clinical trial registration name and number: : ARTEMIS-HP trial; ID number: NCT00879229.
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Introduction

Laboratory biomarkers approach is becoming a new modality screening to differentiate from cardiac and pulmonary dyspnea where other diagnostic tools are unavailable [1]. B-type natriuretic peptide (BNP) and NT pro-BNP are both useful in order to establish diagnosis and prognosis in patients with congestive heart failure [2,3].

Although their clinical application is accepted for patients with cardiovascular diseases, natriuretic peptides are recently used in respiratory disease, as acute pulmonary embolism and pulmonary hypertension (PH) for an early recognition of right ventricular (RV) overload and hypertension [4,5].

In the setting of interstitial lung disease (ILD) it is often difficult to recognize only by clinical examination PH development: shortness of breath and exercise limitation are common symptoms in both ILD and PH [6]. Physical signs as jugular venous distension, tricuspid murmur regurgitation or fixed splitting of pulmonary heart sound, usually appear during advanced stages of PH and have poorly sensibility. Conversely even mild increase of pulmonary pressure may significantly impair the outcome in patients with ILD. Prevalence of PH in ILD ranges from 14% in initial workup to 60% in end stage patients [7–9]. Because of a specific symptoms, PH diagnosis is often delayed particularly in patients with concomitant pulmonary diseases. Therefore the recognition of pulmonary arterial pressure (PAP) in ILD patients is very important for clinical assessment and diagnosis. Moreover, poor correlation was reported between lung function impairment and PH severity: in two different studies the diffusing capacity of the lung for carbon monoxide (DLCO) < 40%, the percent of predicted forced vital capacity (FVC%) and the ratio FVC/DLCO % revealed poor performance to detect PH in this setting [9,10]. Restrictive lung dysfunction is not related to the presence of PH, whereas the need for oxygen supplementation and severe exercise limitation with early oxygen desaturation have been linked to increased pressure [11]. Although the current gold standard for PH assessment is invasive right side catheterization, this procedure should be performed only in specific setting for the evaluation of lung transplantation and when gas exchange abnormalities are disproportionate to ventilation defect [12]. RHC should be executed after PH identification by non invasive methods. However the optimal diagnostic algorithm to assess PH in ILD remains to be elucidated; currently, the most common methods is trans-thoracic echocardiography showing a good correlation with invasive measurement. Nevertheless, in some patients it cannot be performed because poor acoustic window and awkwardness to detect a reliable PAP measurement [13,14]; therefore the prognostic value of Echocardiography, in patients awaiting lung transplantation has been debated because of a mild correlation with RHC measurement [15]. Accordingly whit these findings, we suppose that the combination of pulmonary function tests, echocardiography and BNP could be useful for a foremost recognition of PH in patients with ILD. Considering these findings we would to evaluate: 1-to define the prevalence of PH by echocardiographic and RHC assessment in patients affected by ILD. 2-to evaluated the accuracy of an algorithm including BNP measurement, DLCO and echocardiographic signs to identify PH and RV dysfunction 3- to evaluate BNP and Echo values concordance in relation to right catheterization measurement.

Methods

Study design

Patients were enrolled consecutively from the UO of Respiratory Diseases, Le Scotte Hospital (Siena, Italy) from March 2011 to August 2013. Patients were included with the following characteristics: 1- clinical signs and symptoms (dyspnea, cough, asthenia, poor exercise tolerance); 2-respiratory functional tests alterations (DLCO, Forced espiratory volume [FEV_1], FVC); 3-radiological findings by chest-ray and computed tomography typical for ILD. We evaluated 113 consecutive patients affected by interstitial lung disease. Among these patients we excluded 24 patients for evidence, at echo-doppler, of left ventricular systolic dysfunction or poor acustic window. Patients with echocardiographic PAPs greater than 40 mm Hg (37 patients) underwent to RHC invasive measurement to calculate PAPs, PAP mean and pulmonary vascular resistance (PVR). Written consent was provided by each patient. This sub-study was approved by our hospital's Institutional Review Board and all patients gave their signed informed consent. Patients were recruited from ARTEMIS-HP trial; it was registered and regularly updated in ClinicalTrials.gov with ID number: NCT00879229.

Exclusion criteria

Patients were excluded if they had left ventricular dysfunction, history of pulmonary embolism or obstructive pulmonary diseases, myocardial infarction and renal dysfunction (creatinine > 1.3 mg/dL). We excluded patients with heart failure and preserved systolic function by

E/e^1 ratio analysis (>15). Subjects with sepsis, systemic inflammatory diseases, liver or neoplastic diseases were also excluded.

Laboratory analysis

Within 24 h from enrollment we measured, in all patients, BNP levels. Plasma BNP was measured with Triage BNP Test (Biosite Inc., San Diego, CA, USA); this test is an immunoassay in a single-use plastic cartridge that contains a fluorescently labeled monoclonal antibody against BNP labeled with a fluorescent dye and BNP. There are built-in control features, including control immunoassays, to ensure that the test performs properly and the reagents are functionally active. The test procedure involves the addition of several drops of whole blood or plasma to the sample port on the test device. After addition of the sample, the cells are automatically separated from the plasma via a filter. The sample reacts with fluorescent antibody conjugates within the reaction chamber and flows down the device detection lane by capillary action. The fluorescent antibody conjugates are captured on discrete solid-phase zones resulting in binding immunoassays that are specific for BNP or the control antigens.

Echocardiography

Echocardiography (Hewlett-Packard Sonos 5500 imaging system; Hewlett-Packard Inc) was performed using standard transthoracic windows with a 2.5-MHz transducer. RV dimension was estimated at end-diastole from a right ventricle—focused apical 4-chamber view. Diameter > 42 mm at the base and >35 mm at the mid level indicates RV dilatation. Right atrial (RA) dimension was calculated by the apical 4-chamber view and considered increased for area > 18 cm^2 Tricuspid regurgitation flow was identified by color-flow Doppler techniques and the maximum jet velocity was measured by continuous-wave Doppler in all patients. RA pressure was estimated as 5 mm Hg, 10 mm Hg or 15 mm Hg on the basis of the size and respiratory change of the inferior vena cava (complete collapse, 5 mm Hg; partial collapse, 10 mm Hg; no collapse, 15 mm Hg) [16]. RV systolic pressure was estimated based on the modified Bernoulli equation and was considered to be equal to the Pulmonary systolic Arterial Pressure (PAPs) in the absence of right ventricular outflow obstruction. End-Diastolic Pulmonary Arterial Pressure (PAPd) can be estimated from the velocity of the end-diastolic pulmonary regurgitant jet using the modified Bernoulli equation. Once systolic and diastolic pressures are known, mean pressure may be estimated by the standard formula mean PA pressure = 1/3(PAPs) + 2/3(PAPd).

To obtain Tricuspid Annular Plane Systolic Excursion (TAPSE) the apical four chamber view was used, and an M-Mode cursor was placed through the lateral tricuspid annulus in real time. Offline, the brightness was adjusted to maximize the contrast between the M-Mode signal arising from the tricuspid annulus and the background. TAPSE was measured as the peak excursion of the tricuspid annulus (millimeters) from the end of diastole to the end of systole, with values representing TAPSE being averaged over three to five beats [17]. We did not calculated pulmonary vascular resistance by echo because its estimation is not adequately established to be recommended for routine. Therefore, Current Guidelines do not include noninvasive measurement as a substitute for the invasive evaluation [18].

Echocardiography tapes were reviewed by a cardiologist blinded to other results.

Other investigations

Pulmonary function testing was performed in all patients (and predicted values were calculated according to the American Thoracic Society (ATS) and the European Respiratory Society (ERS) guidelines [19—22]. Lung volumes (constant volume body plethysmograph), spirometric volumes and DLCO were measured.

Invasive measurement

Patients with echocardiographic PAPs greater than 40 mm Hg underwent to RHC in the heamodynamic unit. The RHC was performed with a flow-directed balloon-tipped pulmonary artery catheter leveled to the mid-axillary line and advanced to the pulmonary capillary wedge position. We measured the Pulmonary Artery Pressure (systolic, diastolic, and mean), pulmonary artery WEDGE pressures and Pulmonary Vascular Resistance [23].

Endpoints

1- to define the prevalence of PH by echocardiographic and RHC assessment in patients affected by ILD; 2- to evaluate the sensitivity and accuracy power of the Functional Lung test Echocardiographic and BNP assessments (FLEB) Score in detecting PH; 3- to evaluate BNP and Echo values concordance in relation to RHC measurement of PH, defined as PAP mean > 25 mm Hg.

Statistical analysis

Continuous variables are expressed as mean (±standard deviation or Confidence Interval) and compared using Student's T-test for independent groups if normally distributed; normality was assessed by the Kolmogorov—Smirnov test. Analysis of variance was done by Levene's test, and if it was breached the Welch's correction was used. Spearman's rho correlation coefficient was calculated to determine the relationship among BNP levels, echocardiographic parameters, invasive measurements and DLCO. We also made Receiver Operating Characteristic (ROC) curve analysis to assess the ability of BNP levels, echocardiographic parameters (PAPs, PAP mean and TAPSE) and DLCO to detect PH. For all significant parameters on the ROC Curve analysis we performed logistic regression analysis. We also evaluated the relationship among the score found and the invasive measurements using the concordance analysis and Cohen's "*k*" index. All reported probability values were two-tailed, and a p value <0.05 was considered statistically significant. Statistical analysis was performed using the SPSS 20.0 statistical software package (SPSS Inc., Chicago, IL, USA).

Results

Patients' characteristics

89 patients (age 62 ± 11; 46 males) met the inclusion criteria. ILD diagnoses included: Idiopathic Pulmonary Fibrosis (IPF; n = 63), Sarcoidosis (n = 11), Systemic sclerosis (n = 2), Extrinsic Allergic Alveolitis (EAA; n = 2), smoking-related interstitial lung disease or drug-related interstitial fibrosis (n = 10) and Langherans' cell hystiocitosis (n = 1). In this population there were: 24% of patients with hypertension, 48% of patients with diabetes, 30% of patients with dyslipidemia and 74% with osteoporosis. 37 of these patients showed PAPs≥40 mm Hg, 23 subjects with PAP mean ≥ 25 mm Hg and 25 subjects with right ventricular dysfunction (TAPSE ≤16 mm). All patients with PAPs >40 mm Hg underwent RHC to measure invasive parameters PAPs, PAPmean, WEDGE pressure and PVR. Of 37 catheterized patients, 31 were affected by PH, defined as PAPmean >25 mm Hg. BNP mean in all population was of 60 pg/mL [CI: 34—87]. (Table 1)

- **BNP and RV parameters:** In patients with PAPs > 40 mm Hg, PAPd > 20 mm Hg and with PAP mean > 25 mm Hg, BNP levels were significantly increased compared to patients without pulmonary hypertension (157 ± 96 vs 16 ± 14 pg/ml p = 0.004), PAPd > 20 mm Hg (201 ± 120 vs 28 ± 17 pg/mL p = 0.001) and PAP mean < 25 mm Hg (124 ± 88 vs 23 ± 18 pg/ml p < 0.001). BNP was also significantly increased in patients with right ventricular dysfunction (TAPSE ≤16 mm) (145 ± 104 vs 26 ± 21 pg/ml p < 0.001) and dilatation of RV (End-Diastolic diameter ≥ 38 mm) (175 ± 119 vs 27 ± 20 pg/ml p < 0.001).
- **BNP and RHC measurement:** In patients with invasive PAPs > 40 mm Hg and with invasive PAP mean > 25 mm Hg, BNP levels were significantly increased respect patients with invasive PAPs <40 mm Hg (165 ± 112 vs 29 ± 14 pg/ml p < 0.02) and with invasive PAPmean < 25 mm Hg (194 ± 133 vs 37 ± 29 pg/ml p < 0.005). In patients with Wedge pressure ≥14 mm Hg, BNP levels were significantly higher than patients with Wedge pressure <14 mm Hg (199 ± 153 vs 54 ± 39 pg/mL; p = 0.01). (Table 2)

Correlation among echocardiographic, pulmonary, laboratory tests and invasive measurements

In order to evaluate the relationship between echocardiographic parameters, pulmonary function test parameters and BNP levels we used Spearman' rho correlation coefficient. There were positive significant correlations among BNP and RV DTD (r = 0.56; p < 0.001), BNP and right atrial area (r = 0.45; p = 0.005), BNP and PAPs (r = 0.55; p < 0.001), BNP and PAP mean (r = 0.82; p < 0.001), BNP and ICV diameter (r = 0.37; p < 0.001). An inverse significantly correlation was found between BNP and TAPSE (r = −0.57; p < 0.001). The correlations between BNP and pulmonary functional test parameters (DLCO, TLC and FVC) were not significant.

Table 1 Baseline characteristics of all patients with ILD included in our study. Abbreviations: BNP: B-type Natriuretic Peptide; DLCO: Diffusing Lung capacity for Carbon Monoxide; FE: Ejection Fraction; FVC: Forced Vital Capacity; ILD: Interstitial Lung Disease; PAPd: End-Diastolic Pulmonary Arterial Pressure; PAPs: Pulmonary systolic Arterial Pressure; PAPm: Pulmonary Arterial Pressure mean; PVR: Pulmonary Vascular Resistance; TAPSE: Tricuspid Annular Plane Systolic Excursion; TLC: Total Lung Capacity.

Number of the patients	89
Age (year):	62 ± 11
Weight (Kg):	75 ± 15
Height (cm):	164 ± 9
Gender (n):	
Male	46
Female	43
Risk factors and comorbidity (%):	
Hypertension	25.8%
Diabetes	48.3%
Dyslipidemia	30.3%
Osteoporosis	74.8%
Interstitial Lung Disease (n):	
Pulmonary idiopathic fibrosis	63
Sarcoidosis	11
Extrinsic allergic alveolitis	2
Systemic sclerosis	2
Langherans cell hystiocytosis	1
Other	10
Lung diseases' treatment (%)	
Corticosteroids therapy	93
Immunosuppressive therapy	18
Echocardiographic parameters	
FE (%)	56 ± 3
End-diastolic ventricular diameter (mm)	36 ± 7
Right atrium area (cm^2)	20 ± 3
Caliber Inferior Cave vein (mm)	17 ± 5
PAPs (mm Hg)	36 ± 10
PAPs ≥ 40 mm Hg (n)	37
PAPd (mm Hg)	17 ± 7
PAPmean (mm Hg)	24 ± 8
PAP mean ≥ 25 mm Hg (n)	23
TAPSE (mm)	21 ± 4
TAPSE ≤ 16 mm (n)	25
Invasive measurements (37 patients)	
PAPs (mm Hg)	45 ± 14
PAP m (mm Hg)	25 ± 10
WEDGE pressure (mm Hg)	12 ± 5
PVR (hru)	3.21 ± 2.20
Pulmonary hypertension defined as invasive PAPmean ≥ 25 mm Hg (n)	31
BNP (pg/mL)	60 [34—87]
Pulmonary functional test parameters (%)	
DLCO	55 ± 25
TLC	91 ± 28
FVC	79 ± 27

In the subgroup submitted to right cardiac catheterization (n. 37) we found a significant correlation between PAPs and PAPm calculated with both methods (r = 0.50 and r = 0.43 p < 0.001 and p < 0.01 respectively). (Fig. 1)

Table 2 T-test evaluating BNP value changes relation to echocardiographic and invasive parameters. Abbreviations: BNP: B-type Natriuretic Peptide; PAPd: End-Diastolic Pulmonary Arterial Pressure; PAPs: Pulmonary systolic Arterial Pressure; PAPm: Pulmonary Arterial Pressure mean; TAPSE: Tricuspid Annular Plane Systolic Excursion.

All patients (89)				
Echocardiographic parameters	**BNP mean (pg/mL)**	**Echocardiographic parameters**	**BNP mean (pg/mL)**	**p-values**
PAPs $<$ 40 mm Hg	16 $\pm$ 14	PAPs $\geq$ 40 mm Hg	157 $\pm$ 96	0.004
PAPd $<$ 20 mm Hg	28 $\pm$ 17	PAPd $\geq$ 20 mm Hg	201 $\pm$ 120	0.001
PAP mean $<$ 25 mm Hg	23 $\pm$ 18	PAP mean $\geq$ 25 mm Hg	124 $\pm$ 88	0.001
End diastolic diameter $<$ 38 mm	27 $\pm$ 20	End diastolic diameter $\geq$ 38 mm	175 $\pm$ 119	0.001
TAPSE $>$ 16 mm	26 $\pm$ 21	TAPSE $\leq$ 16 mm	145 $\pm$ 104	0.001
Catheterized patients (37)				
Invasive measurement	**BNP mean (pg/mL)**	**Invasive measurement**	**BNP mean (pg/mL)**	**p-values**
PAPs $<$ 40 mm Hg	29 $\pm$ 14	PAP $\geq$ 40 mm Hg	165 $\pm$ 112	0.02
PAP mean $<$ 25 mm Hg	37 $\pm$ 29	PAP mean $\geq$ 25 mm Hg	194 $\pm$ 133	0.005
WEDGE pressure $<$ 14 mm Hg	54 $\pm$ 39	WEDGE pressure $\geq$ 14 mm Hg	199 $\pm$ 153	0.01

Therefore vascular pulmonary resistance significantly correlates with both PAPs and PAP mean measured non invasively (r = 0.49 and r = 0.44 respectively). TAPSE values were related to all invasive measurement (r = −070 for PAPs r = −0.61 for PAPmeam, r = −055 for wedge r = −044 for VPR).

Diagnostic prediction for PH

The Roc Curve analysis showed that BNP (AUC 0.85; [CI: 0.74—0.96]; p = 0.001), PAP mean (AUC 0.90; [CI: 0.81—0.99]; p = 0.001) and PAPs (AUC 0.84; [CI: 0.72—0.97]; p = 0.001) were all able to detect PH; lower values of DLCO$<$40% (AUC 0.73; [CI: 0.60—0.86]; p = 0.005) and TAPSE $\leq$16 mm(AUC 0.84; [CI: 0.73—0.96]; p = 0.001) also determined PH. (Fig. 2). A cut-off BNP value $\geq$50 pg/ml, recognized patients with PH, with good sensitivity (75%) and good specificity (80%).

Regression analysis of the above parameters confirmed that echocardiographic measurements (PAPs, PAP mean and TAPSE), BNP values and DLCO kept the same trend. (Table 3)

FLEB score index

On the basis of our findings, we built an algorithm including PAPs$\geq$40 mm Hg, PAP mean $\geq$ 25 mm Hg, TAPSE$\leq$16 mm, BNP $>$ 50 pg/ml and DLCO $<$ 40% giving 1 point value for each parameter to validate these non invasive parameters for PH detection measured by RHC. We choose an arbitrary cut-off more than 3 to establish the potential diagnostic value of this score for PH prediction: patients exceeding 3 points demonstrated an excellent concordance with invasive measurements (concordance: 0.964; Cohen's K index: 0.825). (Table 4) For these reasons we would purpose a screening test before to submit ILD patients to RHC. (Fig. 3)

Discussion

- The prevalence of PH in ILD ranges from 14 to 50 %, depending on the status of disease, fibrosis typology, and timing evaluation [7—9]. The measurement of pulmonary pressure is determinant to optimize outcome and possibly to begin a specific treatment. Clinical manifestations of PH are often equivocal, and they could be

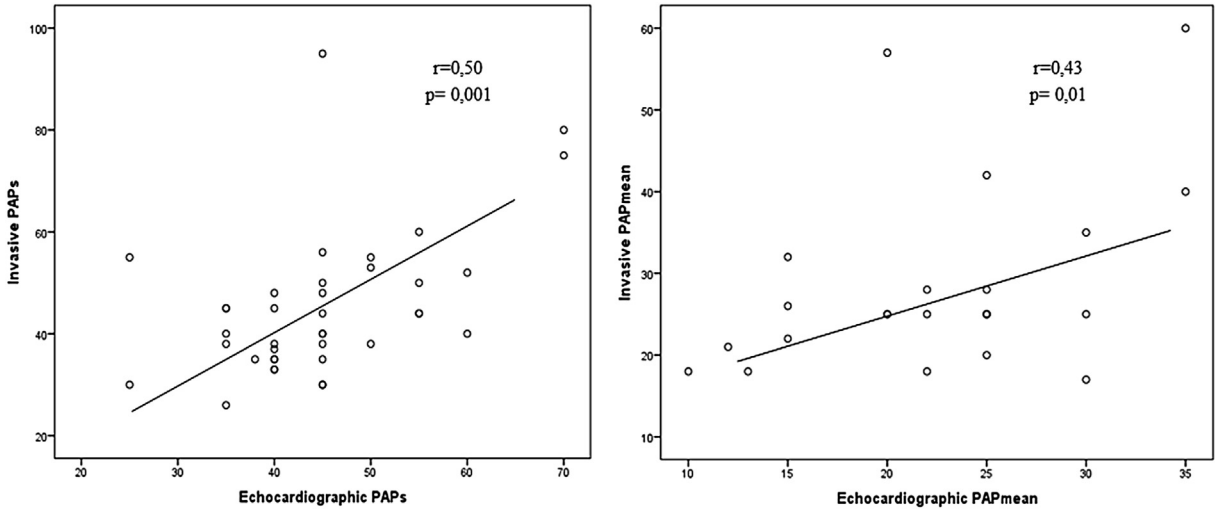

Figure 1 Spearman's rho correlation between PAPs and PAPm assessed by invasive and echocardiographic methods. Abbreviations: PAPs: Pulmonary systolic Arterial Pressure; PAPm: Pulmonary Arterial Pressure mean.

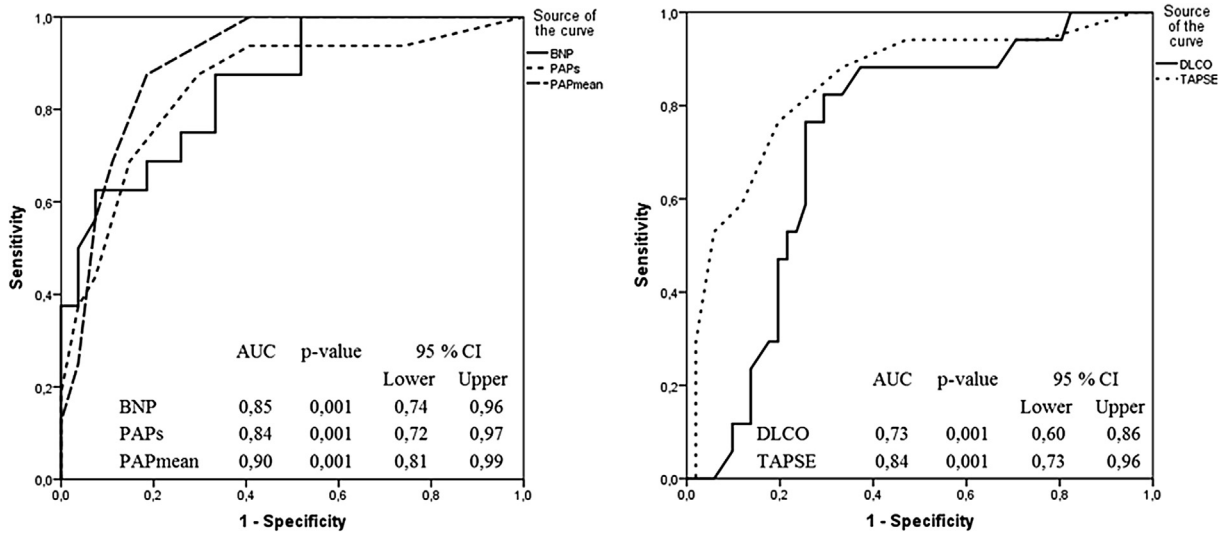

Figure 2 Receiver Operating Characteristic (ROC) curve analysis in predicting patients with PH. Abbreviations: AUC: Area Under the Curve; BNP: B-type Natriuretic Peptide; CI: Confidence Interval; DLCO: Diffusing Lung capacity for Carbon Monoxide; PAPs: Pulmonary systolic Arterial Pressure; PAPm: Pulmonary Arterial Pressure mean; PH: Pulmonary Hypertension; TAPSE: Tricuspid Annular Plane Systolic Excursion.

confused with other cardiac and pulmonary diseases symptoms and signs. For these reasons, current European Society of Cardiology (ESC)/European Respiratory Society (ERS) guidelines recommend an echocardiogram execution every year [24]. Furthermore, the guidelines state that an annual echocardiographic screening ''may be considered'' in asymptomatic patients; a suggestion that is not substantiated by clinical data, but indeed, by expert opinion [25,26]. Our Findings confirm an high prevalence of PH in patients affected to ILD (28%) and that most of patients screened by echocardiography revealed increased pulmonary pressure.

Although this data is in accordance with previous reports, several unanswered questions remain to be elucidated: which populations should be screened, which tools should be used, which diagnostic protocol is really able to better predict PH recognition. Several non invasive protocols have been recently purposed with different results and opinions.

Table 3 Logistic regression analysis evaluating ability of echocardiographic parameters (PAPs, PAPm and TAPSE), DLCO and BNP to detect PH. Abbreviations: BNP: B-type Natriuretic Peptide; CI: Confidence Interval; DLCO: Diffusing Lung capacity for Carbon Monoxide; OR: Odds Ratio; PAPs: Pulmonary systolic Arterial Pressure; PAPm: Pulmonary Arterial Pressure mean; PH: Pulmonary Hypertension; TAPSE: Tricuspid Annular Plane Systolic Excursion.

	Or [CI]	p-value
BNP $>$ 50 pg/mL	12.78 [3.83—22.60]	**0.001**
PAPs $\geq$ 40 mm Hg	20.08 [4.29—46.61]	**0.001**
PAP mean $\geq$ 25 mmHg	9.33 [1.13—26.69]	**0.03**
TAPSE $\leq$ 16 mm	7.80 [1.68—16.06]	**0.009**
DLCO $<$ 40%	6.25 [1.32—17.43]	**0.02**

Some authors [27,28] believe that functional respiratory tests are reliable tools to recognize PH, however recent double blind controlled studies demonstrated that a high percentage of patients without significant reduction of lung function died [29,30].

Other reports assumed that concomitant use of BNP level measurement and echocardiographic assessment together with six minute walking test and oxygen saturation measurement, could lead with good accuracy to an early detection of PH in patients with ILD. Unfortunately findings of these two single center studies are quite different: Modrykamine et al. [31] concluded that PAPs measured by their model is poorly able to detect PH and invite readers to perform invasively measurement of right systolic pressure; conversely Song et al. [32] suggest that combined laboratory and echo study is a useful and repeatable tool to evaluated PH and establish a prognosis in this setting. A recent consensus document to detect PH in chronic lung diseases suggested to perform right heart catheterization in specific subgroups of patients in case of: 1-evaluation for lung transplantation; 2-clinical worsening disproportionate to ventilation impairment; 3- prognostic assessment is deemed to be essential; 4-advanced PH is suspected by non

Table 4 Analysis of concordance between FLEB score and invasive diagnosis of PH. Abbreviations: FLEB: Functional Lung test, Echocardiographic and BNP assessments; PH: Pulmonary Hypertension.

	Patients with PH	Patients without PH
Patients with FLEB score $\geq$3	**29**	**0**
Patients with FLEB score $<$3	**2**	**6**

Concordance Index: 0.964.
Cohen's "k" index: 0.825.

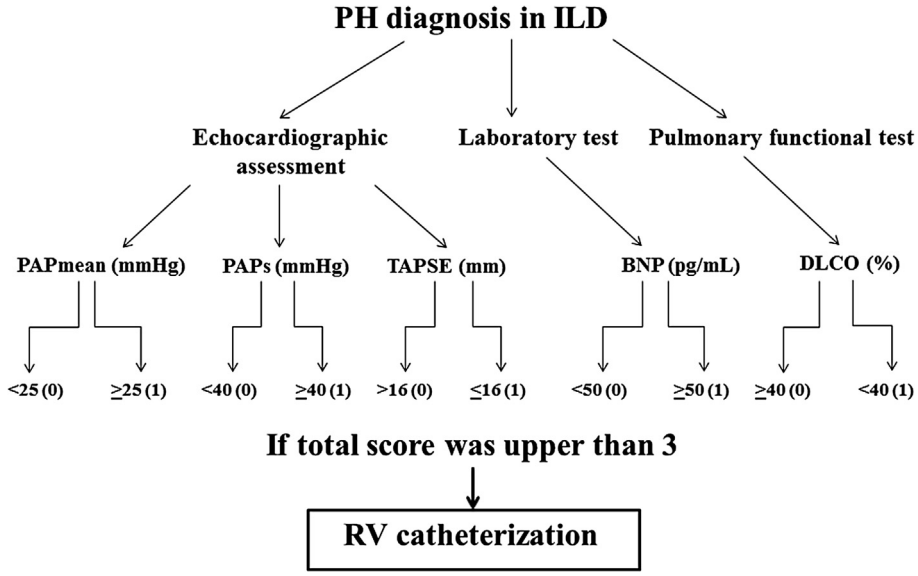

Figure 3 Algorithm model for HP diagnosis in ILD. Abbreviations: BNP: B-type Natriuretic Peptide; DLCO: Diffusing Lung capacity for Carbon Monoxide; ILD: Interstitial Lung Disease; PAPs: Pulmonary systolic Arterial Pressure; PAPm: Pulmonary Arterial Pressure mean; PH: Pulmonary Hypertension; RV: Right Ventricle; TAPSE: Tricuspid Annular Plane Systolic Excursion.

invasive diagnostic examinations; 5-suspicion of left ventricle dysfunction or pulmonary artery embolism that could alter data interpretation. In all other contexts Authors recommended echodoppler and Natriuretic Peptides measurement for initial non invasive diagnosis [9]. Despite recent improvements in diagnostic tools to detect PH in ILD, the methods used routinely in clinical practice have limitations and it may be that a combination of screening tools or parameters will be required to improve the sensitivity and selectivity of current screening programs. As previously revealed, our findings showed that BNP is significantly increased in patients showing PH and RV dysfunction by non invasive and invasive diagnostic tests (echodoppler and right catheterization measurement) [33]: patients showing PH or RV dysfunction evidenced higher BNP values when compared with patients who experienced normal pressure and RV function. A cutoff BNP value more than 50 pg/ml revealed a good accuracy to identify pulmonary pressure increase. Recently a good correlation between hemodynamic and echocardiographic measurements has been evidenced in the ILD patients [34]. Invasive data performed after echocardiographic screening confirmed this analysis. The current protocol adding DLCO, natriuretic peptide to echo measurement appears a good diagnostic algorithm for patients with ILD, so to address and screen those for RCH. Moreover our findings are confirmatory respect to previous reports evaluating the role of BNP in ILD [33].

- On the opposite, poor correlation between BNP and functional respiratory test has been revealed. Only DLCO evidenced an inverse correlation with BNP. Univariate analysis including lung test and risk factors, confirmed that BNP and DLCO are the only two factors for PH prediction. The latter results are not surprising because they reflect the different development of lung fibrosis compared to PH. The two pathophysiological processes have different development and course as demonstrated by the lack of relationship between functional lung parameters PAP and pulmonary resistances.

Although specific studies about interstitial lymphoproliferation and vascular alteration are lacking, there is a general consensus that lung parenchymal remodeling with accompanying hypoxia is the cause of arteriolar vessels decreased capillarity and increase in pulmonary vascular resistance. Lung parenchymal disease is a primary trigger for vascular remodeling and progressive obstruction but it occurs independently of lung impairment [35]. Given the high vasoactive properties, it has been postulated that at least 80% of structural lung lost would lead to PH. A vascular theory directly involving arterial district has been evocated: an increased oxidative stress is the potential trigger for fibroproliferation and vasoconstrictive processes [36,37]. Increased extracellular matrix is associated with reduced synthesis of guanylyl-cyclase and the consequent reduction in nitrix oxide signal transduction. Natriuretic peptide may be partly involved in this process; parenchymal alteration involves tissue natriuretic peptide receptors (NPR) with disruption and downregulation [38].

All these potential factors could explain the good correlation among BNP and pulmonary pressure measurement, but the lack of correlation with respiratory functional parameters reflecting parenchymal status.

The importance of BNP measurement in ILD has been recently demonstrated not only for diagnostic screening but also for risk evaluation: Song et al. [31] showed that a combination of BNP level and non invasive PAPs measurement provide a better prediction of mortality respect to the echo parameter alone. Similarly, Corte et al. [39] demonstrated that elevated BNP concentration and pulmonary vascular resistance levels were independently associated to increased mortality. Nevertheless, previous report did not support the contention that pro BNP could be an reliable

marker of PH in patients with parenchymal lung disease [39]. Although our study did not provide prognostic data, it is a confirmation of the two more recent studies and it evidenced the accuracy of echodoppler examination for PAP estimation compared with the invasive measurement [31,34]. Therefore we analyzed the role of RV dysfunction with respect to BNP level showing a clear correlation between RV overload and systolic performance and BNP level. We considered it important to establish whether dichotomous BNP values provides diagnostic information on PH: patients with BNP levels above the threshold of 50 pg/ml revealed good specificity for PH and RV dysfunction, confirming that BNP as a dichotomous variable could potentially provide additional diagnostic information over echocardiography. Considering that a universal screening method routinely applied in clinical practice does not exist, on the basis of our results we can purpose an algorithm considering BNP assay before echocardiography for a better screening of PH in patients with ILD. Our findings extend the literature by offering a combined evaluation of BNP and echocardiographic measurements of RV dysfunction as predictors of PH. Finally the same dichotomous approach should be used for Echo measurement and DLCO into an algorithm Score including all parameters to better stratify patients with PH needing invasive RHC study: based upon our data features, a score more than 3 points should be a good indicator for PH. If confirmed in a larger population these parameters and cutoff values could be used to identify patients to be submitted to RHC invasive measurements.

Limitations

Our study was limited by its retrospective design and patient selection evaluated in a single tertiary center, therefore this study cannot exactly reflect the all ILD patients. A wide range of disease severity was evaluated, BNP and echocardiography were performed routinely on new referrals. The invasive study was conducted only in patients revealing PH at Echo examination so the exact BNP accuracy in the population unsubmitted to cardiac catheterization cannot be confirmed. We suggest that the resultant range of disease severity and suspicion of PH involvement both reflect real-life clinical practice, and is a representative population in which to explore proof of concept outcome analyses. We did not calculated all the noninvasive echo parameters recommended for PH diagnosis as pulmonary artery dilatation, acceleration time of RV outflow tract. However, our aim would be provide a standard simple method able to identify PH. Our data cannot definitively excluded patients with PH secondary to heart failure with isolated diastolic dysfunction, but an analysis of pulsed Doppler transmitral flow and tissue doppler flow has been performed and we excluded subjects with more advanced dysfunction excluding those with $E/e^1 > 15$. Exact clinical utility with reference to unselected ILD cases cannot be extrapolated from our data. The algorithm construction purposed by combining BNP and echocardiographic thresholds, was hampered by low subgroup numbers. Besides, even if our score can be considered an accurate index to detect PH, it cannot be applied for outcome assessment. Prospective larger studies with follow-up observation are required to further delineate the relative importance of these prognostic markers alone and in combination, and before these markers can be widely used in clinical practice. Despite these limitations, our study is the first attempt to evaluate the diagnostic role of echocardiography and BNP levels in patients with ILD and associated PH.

Conclusions

Our data suggest that an algorithm including BNP DLCO and echocardiography findings is an useful and repeatable tools for PH detection in patients with ILD. This protocol could help in early identification of PH and it should be applied in clinical practice as a preliminary screening for invasive hemodynamic study selection. The current results need to be confirmed in a larger sample size and validated by follow-up data.

Conflict of interests

None declared.

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GMR has given substantive intellectual contributions to a published study. He has given substantial contributions to conception and design. He has given final approval of the version to be published. He is the guarantor of the paper, taking responsibility for the integrity of the work.

BC has given substantial contributions to acquisition of data and interpretation of data.

PR has given substantial contributions to acquisition of data and interpretation of data.

RMR has given substantial contributions to acquisition of data and interpretation of data.

MP has given contributions to acquisition of data.

CDT has performed echocardiography examination.

GDC has revised English language of paper.

BF has analyzed the blood samples to measure BNP.

RN has been involved in drafting the manuscript or revising it critically for important intellectual content.

AP has been involved in drafting the manuscript or revising it critically for important intellectual content. He has given substantial contributions to conception and design. He has given final approval of the version to be published.

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