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Increased liver stiffness in idiopathic pulmonary fibrosis: a pilot study

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Introduction

Fibrosis of epithelial parenchymal organs and end-stage organ failure represent the final common pathways of many chronic diseases and are major determinants of morbidity/mortality worldwide. Comparison studies of pulmonary, hepatic and renal fibrosis have identified several common mechanisms, including a critical role for the cytokine transforming growth factor-beta and the generation of reactive oxygen species (1). Idiopathic pulmonary fibrosis (IPF) is a progressive and devastating illness with a mean survival of less than 5 years from the onset of symptoms. IPF epidemiology is on the rise but underlying pathogenetic mechanisms are not clearly elucidated (2). At present, IPF is considered as an epithelial/fibroblastic disorder resulting from an unabated continuation of the repair process in response to recurrent episodes of epithelial injury (3). There is evidence that oxidative stress may be a key player in this scenario (4), conversely, the role of inflammation still remains largely controversial while emerging data suggest the immune system involvement (5). IPF natural history may also differ among patients due to a significant heterogeneity in disease behavior. As a consequence, any effort tailored at the characterization of IPF sub-phenotypes is mandatory.

To address whether the liver may be involved, apart from the lung, in the challenging IPF clinical picture, the aim of the present study was to evaluate liver stiffness values in clinically stable IPF patients. Novel non-invasive tests such as transient ultrasound elastography (TUE) are widely used to stage liver fibrosis as an alternative to liver biopsy, and this technology has recently been approved in the US (6). TUE has been proposed as a possible tool for diagnosis and monitoring of non-significant fibrosis or severe fibrosis/cirrhosis in patients with chronic hepatitis C virus (HCV) infection. Liver stiffness values have also been significantly correlated with histopathological staging of liver biopsy, with excellent accuracy in distinguishing absent/mild and advanced fibrosis (7).

Case series report

Twenty-nine consecutive Italian IPF patients were enrolled between June 2012 and December 2013. Diagnostic preliminaries included clinical history, physical examination, routine and autoimmunity blood tests, lung function testing, arterial blood gases analysis, incremental thin-section high resolution computed tomography scan (HRCT) of the

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thorax and echocardiography. The 6 minute walk test and fiberoptic bronchoscopy were performed in selected cases. Disease diagnosis was made according to the recent American Thoracic Society/European Respiratory Society consensus statement (2). Demographics and clinical features are reported in Table 1. Fifteen age- and sex-matched healthy volunteers were recruited among hospital staff as control group. Lung function parameters are reported in Table 2. The composite physiologic index (CPI) (8), the GAP score (9) and the Kazerooni visual qualitative score of disease extension on HRCT scan (10)

Table 1. Demographics and clinical characteristics of IPF patients

Parameter	IPF patients	Controls
Gender	24M/5F	12M/3F
Age (years)	67±8.3	64±3.9
BMI (Kg/m^2)	28±1.7	27±1.9
Smokers/former smokers/never smokers	0/19/10	0/5/10
Comorbidities:		
- systemic arterial hypertension	8 (27.5%)	
- chronic ischemic heart disease	6 (20%)	
- type II diabetes	6 (20%)	
- combined pulmonary emphysema	2 (7%)	
- GERD	10 (34%)	
- pulmonary arterial hypertension*	4 (14%)	
Treatments for IPF:		
- pirfenidone (2403 mg/day), and/or	9 (31%)	
- NAC (1800 mg/day)	7 (24%)	
- other (experimental trial drugs)	2 (7%)	
- no therapy	15 (52%)	
Long-term oxygen therapy	14 (48%)	-

Data are expressed as mean±SD or as absolute number (percentage)
Abbreviations: BMI= body mass index; GERD= gastro-esophageal reflux disease; NAC= N-acetylcysteine.
* Likely/possible pulmonary hypertension was diagnosed by means of echocardiography according to recent guidelines (17) with an estimated mean systolic pulmonary artery pressure±SD of 30.5 ± 6.6 mmHg.

Table 2. Respiratory function parameters and thorax HRCT scoring in IPF patients

Parameter	IPF patients	Controls
pO_2 (mmHg) (21% FiO_2 at rest)	69.5±13.6; 71.6 [63.8-75.3]	-
% sO_2 (21% FiO_2 at rest)	92.3±5.7; 95 [89.9-94.7]	98±1.6; 97 [96-100]
FVC (L)	3.1±4.0; 2.4 [1.3-4.9]	3.5±0.8; 3.4 [2.7-4.0]
FVC (% predicted)	69.8±18.7; 69 [61.5-78.1]	98±12.5; 92 [86-108]
TLC (L)	3.5±1.3; 3.3 [2.7-4.2]	5.2±0.8; 5 [4.5-5.5]
TLC (% pred)	60.6±18.7; 61 [49.8-71.4]	86±38; 85 [83-88.5]
D_LCO_{sb} (Hb-adjusted) (ml/min/mmHg)	9.3±4.5; 8.7 [7.1-11.5]	24±6; 24 [19-28]
D_LCO_{sb} (Hb-adjiusted) (% predicted)	41.9±17.8; 41 [33.2-50.6]	86±5; 84.5 [81-88]
6 MWT distance*, m	405±139; 432 [325-492]	-
CPI	50.4±13.3; 53.4 [42.7-56.1]	-
GAP score	I (14); II (12); III (3)	-
Kazerooni score		
Ground glass opacities	2.5±1.8; 2 [1-4]	-
Fibrosis	9.6±4.2; 8 [6-12.2]	-

Data are expressed as mean±SD; median [IQR_{25}-IQR_{75}]
Abbreviations: pO_2= arterial O_2 partial pressure; sO_2= arterial O_2 saturation; FVC= forced vital capacity; D_LCO_{sb}= single breath diffusing capacity for carbon monoxide; Hb= haemoglobin; TLC= total lung capacity; 6 MWT= six-minute walk test (* data available for 15 patients);

Table 3. Respiratory function parameters and thorax HRCT scoring in IPF patients according to liver stiffness

Parameter	IPF patients with no increased liver stiffness	IPF patients with increased liver stiffness
pO_2 (mmHg)(21% FiO_2 at rest)	68.4±11.3; 72.3 [64-75.8]	73.2±14.3; 76 [69.9-81]
FVC (% pred)	69.4±19.8; 66 [52-82.5]	65.5±15.4; 64 [57.5-73.2]
TLC (% pred)	58±17.3; 57.5 [45.5-67.7]	60.7±15.7; 58.5 [52.7-69]
D_LCO_{sb} (Hb-adjiusted) (% pred)	42.7±12.2; 44 [35-47]	53.7±22.7; 51.2 [43.5-65.5]
6 MWT distance*, m	443±78; 464 [412-490]	377±133; 396 [320-462]
sPAP, mmHg	31.5±6.2; 30 [26-35]	28.7±7.4; 25 [25-30]
CPI	51.3±11.4; 53.6 [46.7-56.8]	44.1±18.2; 43.8 [31-54.1]
GAP score	I (8), II (7); III (2)	I (6), II (5); III (1)
Kazerooni score		
Ground glass opacities	2.3±2; 2 [1-3.75]	2.5±1.6; 2 [2-3]
Fibrosis	9.7±3.8; 9.5 [6.5-12.7]	7.8±2.8; 7 [6-8]
Any treatment (n)		
- pirfenidone (2403 mg/day)	5	4
- NAC (1800 mg/day)	3	4
- other (experimental trial drugs)	1	1
- no therapy	9	6

Data are expressed as mean±SD; median [IQR_{25}-IQR_{75}]
Abbreviations: pO_2= arterial O_2 partial pressure; sO_2= arterial O_2 saturation; FVC= forced vital capacity; D_LCO_{sb}= single breath diffusing capacity for carbon monoxide; Hb= haemoglobin; TLC= total lung capacity; 6 MWT= six-minute walk test (* data available for 15 patients); CPI= composite physiologic index; sPAP= systolic pulmonary artery pressure. Comparisons were not significant.

are also reported for IPF patients. The local Ethics Committee approved the study and all participants provided written informed consent. Liver imaging analysis and TUE were realized under fasting conditions by two independent operators by means of conventional ultrasound examination (Philips iU 22, Eindhoven, The Netherlands) and FibroScan (EchoSens, Paris, France), respectively. Abdominal ultrasonography was performed with a convex multifrequency transducer of 1-5 MHz to detect any sign suggestive of liver cirrhosis (irregular surface, coarse texture, attenuated hepatic veins) and/or of portal hypertension (presence of abdominal collaterals, splenomegaly), ascites and to exclude hepatic focal lesions. Liver stiffness measurements (LSMs) were realized as follows: up to ten acquisitions were performed on each patient. Success rate was calculated as the ratio of the number of successful acquisitions over the total number of acquisitions. The median value of successful measurements was kept as representative of the liver stiffness. Only LSMs obtained with a success rate of at least 60% were considered reliable. The median value of successful measurements was considered representative for the liver stiffness in a given patient if the interquartile range of the examinations was less than 30% of the median (11). Serum levels of alanine aminotransferase, aspartate aminotransferase, total and direct bilirubin, albumin, alpha-fetoprotein, total cholesterol and tryglicerides levels, prothrombin time and concentration, along with hepatitis B virus (HBV) and HCV antibodies were preliminary analyzed in all subjects. HBV or HCV infection and alcohol abuse were exclusion criteria. Total IgG and IgG subsets serum levels were also determined. Increased IgG4 titers were recorded in no cases.

No remarkable alterations of liver-related parameters measured at the serum level were found in the study groups (data not shown). According to the criteria of Quyyum et al. evaluating the degree of liver steatosis on a five-point scale, that is 0 (absent), 1 (minimal), 2 (mild), 3 (moderate), and 4 (severe) based on liver echogenicity (12), mild/moderate steatosis was identified in respectively 1 control subject and 8 IPF cases (6.6% and 27.5%). Severe steatosis was detected in 2 IPF patients (7%), without additional pathologic findings. LSMs were successfully collected in all subjects analyzed. Mean TUE measurements were 5.2±0.4 kPa in the control group as compared to 6.4±2.2 kPa in IPF patients (p=0.02, two tails unpaired t test Welch corrected); to date, higher values were recorded in 11 IPF cases (38%), suggesting increased liver stiffness (8.2±1.9 kPa). Stratification of IPF patients according to liv-

er stiffness values (normal versus increased) is summarized in Table 3. As reported, no substantial differences occurred in terms of lung function parameters, disease and HRCT scoring, treatment or mortality.

Discussion

In the present study, hepatic elastography was used to evaluate the liver stiffness values in clinically stable IPF patients in a real life setting. To our knowledge, there are no available reports about the use of non-invasive methods to early predict the liver involvement in this scenario. TUE has recently been approved in the US and widely used instead of liver biopsy as an alternative tool for diagnosis and monitoring of non-significant fibrosis or severe fibrosis/cirrhosis in patients with chronic HCV infection (6). This preliminary small case series suggests that increased liver stiffness may be present in approximately more than one-third of IPF patients. Unfortunately, liver biopsy was not performed in our study due to ethical reasons: as a consequence, it was not possible to ascertain which histo-pathological components contributed to these findings. Furthermore, although TUE has a high degree of accuracy and reproducibility in prediction of liver fibrosis in the context of HCV infection, there are conflicting reports as to the impact of steatosis on FibroScan measurements. Arena et al. observed no influence of steatosis on TUE (13), whereas Sanchez-Conde et al. (14) and Boursier et al. (15) noted significant associations, especially among patients with high-grade steatosis. This was not the case in our pilot study, as only two IPF patients had a severe degree of steatosis but both of them were recorded to have normal liver stiffness values. Further confounding factors likely affecting liver stiffness were also investigated. To date, clinical and instrumental findings suggestive of right heart insufficiency were carefully excluded in all patients. Not surprisingly, no significant correlations were found between liver stiffness and lung function parameters. Similarly, no association was observed with other disease severity indices, that are the CPI and the Kazerooni score. This may be dependent on the small number of patients enrolled in a single center setting that clearly represents a limitation of our study. Conversely, up to now, the mortality rate was lower among patients with evidence of increased liver stiffness, with this finding not having a clear explanation. Again, our feeling is that such an observation may be not conclusive as comorbidities and longer disease duration cannot be ruled out as outcome determinants in patients with no signs of any liver involvement. Addressing this issue needs longitudinal efforts in larger study populations.

In conclusion, as IPF is still considered a rare disease requiring a multi-disciplinary diagnostic process, we strongly believe that any effort improving knowledge in such a challenging scenario should be encouraged. We do not really believe that IPF is a systemic disease and our preliminary data are not sufficient to support such an hypothesis. To our knowledge, this is the first attempt to incorporate into clinical practice novel methodologies aimed at delineating in a more comprehensive fashion the complex IPF clinical phenotype. Apart from several limitations, it could pave the way to prospective confirmation studies in larger patient samples. The role of genetic factors, including telomere shortening, is also an issue to be investigated (16). The clinical relevance and the likely involvement of additional organs (i.e, kidney, heart) should be addressed as well.

References

1. Kisseleva T, Brenner DA. Fibrogenesis of parenchymal organs. Proc Am Thorac Soc 2008; 5: 338-42.
2. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis, An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011; 183: 788-824.
3. Selman M, Pardo A. The epithelial/fibroblastic pathway in the pathogenesis of idiopathic pulmonary fibrosis. Am J Respir Cell Mol Biol 2003; 29: S93-S97.
4. Bocchino M, Agnese S, Fagone E, Svegliati S, Grieco D, Vancheri C, et al. Reactive oxygen species are required for maintenance and differentiation of primary lung fibroblasts in idiopathic pulmonary fibrosis. PLoS One 2010; 5: e14003.
5. Galati D, De Martino M, Trotta A, Rea G, Bruzzese D, Cicchitto G, et al. Peripheral depletion of NK cells and imbalance of the Treg/Th17 axis in idiopathic pulmonary fibrosis patients. Cytokine 2014; 66: S119-126.
6. Bonder A, Afdhal N. Utilization of FibroScan in clinical practice. Curr Gastroenterol Rep 2014; 16: 372. doi 10.1007/s11894-014-0372-6.
7. Castera L, Forms X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol 2008; 48: 835-47.
8. Wells AU, Desai SR, Rubens MB, Goh NS, Cramer D, Nicholson AG, et al. Idiopathic pulmonary fibrosis: a composite physiologic index derived from disease extent observed by computed tomography. Am J Respir Crit Care Med 2003; 167: 962-9.
9. Ley B, Ryerson CJ, Vittinghoff E, Tomassetti S, Lee JS, Poletti V,

et al. A multidimensional index and staging system for idiopathic pulmonary fibrosis. Ann Intern Med 2012; 156: 684-91.
10. Kazerooni EA, Martinez FJ, Flint A, Jamader DA, Gross BH, Spizamy DL, et al. Thin section CT obtained at 10 mm increments versus limited three-level thin-section CT for idiopathic pulmonary fibrosis: correlation with pathologic scoring. Am J Roentgenol 1997; 169: 977-83.
11. Boursier J, Zarski JP, de Ledinghen V, Rousselet MC, Sturm N, Lebail B, et al. Multicentre group ANRS HC EP23 FIBROSTAR. Determination of reliability criteria for liver stiffness evaluation by transient elastography. Hepatology 2013; 57: 1182-91.
12. Quyyum A, Chen DM, Breiman RS, Westphalen AC, Yeh BM, Jones KD, et al. Evaluation of diffuse liver steatosis by ultrasound, computed tomography, and magnetic resonance imaging: which modality is best? Clin Imaging 2009; 33: 110-5.
13. Arena U, Vizzuti F, Abraldes J, Corti G, Stasi C, Moscarella S, et al. Reliability of transient elastography for the diagnosis of advanced fibrosis in chronic hepatitis C. Gut 2008; 57: 1288-93.
14. Sanchez-Conde M, Montes Ramirez ML, Bellon Cano JM, Caminoa A, Alvarez Rodriguez F, Gonzalez-Garcia J, et al. Impact of liver steatosis on the correlation between liver stiffness and fibrosis measured by transient elastography in patients coinfected with human immunodeficiency virus and hepatitis C virus. J Viral Hepat 2011; 18: e278-283.
15. Boursier J, de Ledinghen V, Sturm N, Amrani L, Bacq Y, Sandrini J, et al. Multicentre group ANRS HC EP23 FIBROSTAR. Precise evaluation of liver histology by computerized morphometry shows that steatosis influences liver stiffness measured by transient elastography in chronic hepatitis C. J Gastroenterol 2014; 49: 527-37.
16. Carulli L, Dei Cas A, Nascimbeni F. Synchronous cryptogenic liver cirrosis and idiopathic pulmonary fibrosis: a clue to telemere involvement. Hepatology 2012; 56: 2001-3.
17. Galiè N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, et al. ESC Committee for Practice Guidelines (CPG) Guidelines for the diagnosis and treatment of pulmonary hypertension: the Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). Eur Heart J 2009; 30: 2493-537.