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Circulating biomarkers of tissue remodelling in pulmonary hypertension

Christian Schumann[1], Philipp M. Lepper[2], Hedwig Frank[1], Roland Schneiderbauer[1], Thomas Wibmer[1], Cornelia Kropf[1], Kathrin M. Stoiber[1], Stefan Rüdiger[1], Lothar Kruska[3], Thomas Krahn[4], and Frank Kramer[4]

[1]Clinic of Internal Medicine II, University of Ulm, Ulm, Germany, [2]Department of Pneumology University Hospital (Inselspital) and University of Bern, Bern, Switzerland, [3]MVZ Dr. Stein und Kollegen, Mönchengladbach, Germany, and [4]Global Biomarker Research, Bayer HealthCare AG, Wuppertal, Germany

Abstract

Objective: Besides persisting high pulmonary arterial pressure and increased pulmonary vascular resistance, remodelling of pulmonary tissues and subsequently the right heart are the key pathomechanisms of pulmonary hypertension (PH). Extracellular matrix maintenance in this context plays a central role.

Methods: We tested the hypothesis that plasma concentration of matrix metalloproteinase (MMP)-2, tissue inhibitor of matrix metalloproteinases (TIMP)-4 and tenascin C (TNC) might be useful as biomarkers for assessing the severity of PH. Therefore, the concentrations of MMP-2, TIMP-4, TNC and N-terminal b-type natriuretic peptide (NT-proBNP) of 36 PH patients were compared with those of 44 age- and gender-matched healthy volunteers. Additionally, lung function, 6-min walk distance and right heart function were assessed.

Results: In PH patients, significantly elevated plasma levels of MMP-2, TIMP-4, TNC and NT-proBNP were detected. In particular, TIMP-4 was significantly increased in patients with higher NYHA classification, and in patients with severe right ventricular hypertrophy.

Conclusion: Monitoring of plasma TIMP-4 and to a lesser extent of MMP-2 and TNC levels in PH patients might help to assess the beneficial effects of PH pharmacotherapy on tissue remodelling.

Keywords: *Biomarkers; tissue remodelling; pulmonary hypertension; matrix metalloproteinase 2; tenascin C; tissue inhibitor of matrix metalloproteinase 4*

Introduction

Pulmonary hypertension (PH) is a potentially life-threatening and progressive disease, characterized by elevation of pulmonary artery pressure (PAP), vascular resistance (PVR), vascular-wall remodelling and thrombosis *in situ* that ultimately leads to right ventricular failure and death (Rubin 1997). The World Health Organization (WHO) classification (Venice classification) of PH consists of five categories in which PH diseases are grouped: (1) pulmonary arterial hypertension (PAH), (2) pulmonary venous hypertension, (3) PH associated with disorders of the respiratory system or hypoxemia, (4) PH caused by thrombotic or embolic diseases (CTEPH), and (5) PH caused by diseases affecting the pulmonary vasculature (Simonneau et al. 2004). The median survival of untreated patients with PAH is 2.8 years with a 5-year survival rate of 34% (D'Alonzo et al. 1991). Among clinical parameters, several variables are associated with poor outcome, e.g. New York Heart Association (NYHA) functional class (FC) of III and IV, elevated PVR, PAP, right atrial pressure (RAP) >20 mmHg, cardiac index (CI) <2.0 l min^{-1} m^{-2}, pulmonary arterial (mixed venous) oxygen saturation (SvO_2) <63%, 6-min walk distance (6-MWD) <380 m under treatment, peak oxygen uptake (VO_2) <10.4 ml kg^{-1} min^{-1} and a maximum systolic blood pressure during exercise

C. Schumann and F. Kramer contributed equally to the manuscript.

Address for Correspondence: Frank Kramer, Bayer HealthCare AG, Global Biomarker Research, Aprather Weg 18a, Wuppertal, Germany. Tel: +49 202 36 5680. Fax: +49 202 36 4944. E-mail: Frank.Kramer@bayerhealthcare.com

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(<120 mmHg) (Sitbon et al. 2002, Wensel et al. 2002). In particular, FC, 6-MWD and cardiopulmonary exercise testing can guide modern goal-oriented therapy in order to detect clinical worsening, adjust treatment and finally improve survival (Hoeper et al. 2005). Although various therapy options are available for the treatment of PAH, there is still a high medical need in this entity. To date, approved drugs for the treatment of PAH are among others phosphodiesterase-5 (PDE-5) inhibitors, endothelin-1 (ET-1) receptor antagonists and prostanoides (Reichenberger et al. 2006, Dupuis & Hoeper 2008, Gomberg-Maitland & Olschewski 2008). Although all available drugs for the treatment of PAH – when applied as mono- or combined therapies – are efficacious in reducing vasomotor tone, they have only limited impact on the cardiopulmonary remodelling processes (Ali et al. 2007, Benza et al. 2007). However, there are some promising approaches to inhibit processes that induce tissue remodelling, e.g. the inhibition of the human neutrophil elastase and blockage of tyrosine kinase signalling (Ali et al. 2007). The role of biomarkers for the assessment of disease severity and response to therapy remains controversial. Numerous soluble biomarkers, such as von Willebrand factor, ET-1, eicosanoid metabolites and others as well as endothelial progenitor cells and circulating endothelial cells have been linked to disease mechanisms and partly to prognosis (Christman et al. 1992, Kawut et al. 2005, Asosingh et al. 2008, Smadja et al. 2009). Atrial natriuretic peptide (ANP) and b-type natriuretic peptide (BNP), which are derived from the atria and ventricles in response to myocyte stretch, (1) reflect pressure overload, (2) are elevated in PAH, (3) correlate with disease progression and (4) may predict mortality (Leuchte et al. 2004). In addition, a decrease of the N-terminal peptide of BNP (NT-proBNP) is associated with response to treatment (Fijalkowska et al. 2006, Suntharalingam et al. 2008). A comprehensive description of the most important PH biomarkers and their potential applications can be found elsewhere (Warwick et al. 2008).

Besides persistently high PVR and PAP, tissue remodelling in the pulmonary circulation and subsequently in the right heart are key pathomechanisms of PH. Proliferation and migration of smooth muscle cells (SMCs) that lead to intimal and medial thickening is partly regulated by mitogenic factors which are released after endothelial damage (Wagenvoort 1981). Fibrotic changes in heart and lung tissue are controlled by the activity of proteolytic enzymes, such as the extracellular matrix (ECM)-degrading metalloproteinases (MMPs) (Polyakova et al. 2004). Endogenous tissue inhibitors of matrix metalloproteinases (TIMPs) tightly control the activity of MMPs. In pathological processes, local TIMP expression can be decreased as well as increased to regulate the enzymatic activity of MMPs. Furthermore MMPs can stimulate the secretion of tenascin-C (TNC), which acts as a strong mitogenic cofactor, leading to SMC proliferation (Tamaoki et al. 2005). It can be assumed that the MMP–TIMP balance and mitogenic factors of the ECM like TNC are crucial in the pathogenesis of PH.

Therefore, determination of sentinel and integral soluble heart and lung-derived biomarkers might help to assess the status of remodelling processes in lung and heart tissue. This study was designed to examine the role of TIMP-4, MMP-2 and TNC in PH patients and to evaluate whether plasma concentrations of these proteins correlate with clinical endpoints and thereby reflect disease severity. If serum concentrations of TIMP-4, MMP-2 and TNC mirror the status of cardiopulmonary tissue remodelling, these biomarkers might help to monitor therapy response especially when patients are treated with non-haemodynamically active anti-remodelling therapies such as multikinase or protease inhibitors. As cardiopulmonary tissue remodelling is a common feature in all forms of PH, we included patients with different etiologies of PH into our study (clinical classes 1–4 of WHO classification).

Materials and methods

Patient population, healthy control individuals and study design

The ethics committee of the University of Ulm approved the study. Patients with clinical stable PH were included. The diagnosis was set in accordance with the current guidelines of the European Society of Cardiology (Galie et al. 2009). During follow-up, all patients had regular control visits in our outpatient department. The enrolment period was between April 2007 and June 2008; all patients gave written informed consent. We analysed plasma samples of 36 PH patients. For details of the patient population see Table 1. In addition blood samples were obtained from 44 healthy blood donors (24 male, 20 female). Age of patients was comparable to the age of the healthy control individuals (healthy control group: 26–78 years, mean 53 years, for age distribution see Figure 3).

Blood sampling

Blood samples were allowed to clot for 30 min and subsequently centrifuged for 15 min at 1000 *g* at 4°C. Supernatants were removed and stored at –80°C until analysis.

Analysis of biomarkers

For the analysis of biomarkers commercially available immunoassays were used. We determined the plasma concentrations of TIMP-4 (DTM400; R&D Systems,

Table 1. Patient characteristics and baseline functional data.

Demographics		
Age (years)		68.5 ± 2.2
Gender, male/female		19/17
Body mass index		27.8 ± 0.9
Current or former smoker* (pack-years)		20 (18.5 ± 4.2)
Never smoker		16
WHO clinical class		
1	IPAH (16), APAH (3)	19
2		2
3		4
4	CTEPH (11)	11
NYHA/functional class		
II		15
III		21
Treatment		
Endothelin receptor antagonists		13
Phosphodiesterase-5 inhibitor		5
Without specific PH-treatment or naive		18
Lung function, cardiopulmonary exercise and haemodynamics		
pO_2	mmHg	63.3 ± 2.3
pCO_2	mmHg	36.1 ± 0.9
TLCO SB	mmol min^{-1} kPa	54.1 ± 4.0
$EqCO_2$		43.5 ± 4.4
AT-VO_2	ml kg^{-1} min^{-1}	11.9 ± 1.1
Peak-VO_2	ml kg^{-1} min^{-1}	13.4 ± 1.1
6-MWD	m	299 ± 21.6
sPAP	mmHg	59.8 ± 4.1
mPAP	mmHg	36.3 ± 2.7
PVR	dyn*s*cm^{-5}	465 ± 54.9
CWP	mmHg	10 ± 0.8
CO	l min^{-1}	4.3 ± 0.15
CI	(l min^{-1}) m^{-2}	2.3 ± 0.1

Values are presented as mean ± SEM.
pO_2, pCO_2, oxygen and carbon dioxide arterial tension; TLCO SB, transfer factor for carbon monoxide diffusion capacity; $EqCO_2$, ventilatory equivalent for carbon dioxide (on exercise); AT-VO_2, oxygen uptake at anaerobic threshold; peak-VO_2, maximum oxygen uptake during exercise; sPAP, systolic pulmonary artery pressure (estimated by echo Doppler); mPAP, mean pulmonary artery pressure (measured by right heart catheterization); PVR, pulmonary vascular resistance; CWP, capillary wedge pressure; CO, cardiac output; CI, cardiac index; WHO clinical class, PH classification Venice 2006 (IPAH, idiopathic; APAH, associated; CTEPH, chronic thrombotic/embolic pulmonary disease).

Minneapolis, MN, USA), MMP-2 (DMP200; R&D Systems), TNC (#27751; IBL, Hamburg, Germany) and NT-proBNP (ECLIA, Modular Analytics E170, Elecsys® Modul; Roche Diagnostics, Basel, Switzerland).

Assays were performed according to recommendations of the manufacturer. The mean minimal detected concentration (MDC) for analytes was 4.9 pg ml^{-1} for TIMP-4, 0.16 ng ml^{-1} for MMP-2 and 0.16 ng ml^{-1} for TNC. The intra-assay precision determined as coefficient of variation (CV) for the TIMP-4 enzyme-linked immunosorbent assay (ELISA) was 4.6% at 1.5 ng ml^{-1} ($n = 20$), for the MMP-2 ELISA was 3.4% at 17.4 ng ml^{-1} ($n = 20$) and for the TNC ELISA was 3.9% at 13.55 ng ml^{-1} ($n = 20$).

Right heart catheterization

Haemodynamic studies were performed at baseline. An 8-F introducer sheath was placed in the right or left internal jugular vein, through which a triple-lumen 7-F Swan-Ganz thermodilution catheter (Edwards Lifesciences, Irvine, CA, USA) was introduced. Heart rate, ECG, systemic arterial pressure and oxygen saturation were measured continuously. Cardiac output (thermodilution method), PAP, mean RAP and pulmonary capillary wedge pressure were assessed. Systemic and pulmonary vascular resistances were calculated using standard equations. Mixed-venous blood samples were drawn and analysed for arterial oxygen saturation with a blood gas analyser (ABL 555; Radiometer, Copenhagen, Denmark).

Echocardiography

At baseline, transthoracic 2D and colour Doppler echocardiography was performed using the echocardiography apparatus with a 2.5-MHz transducer on an ATL HDI 5000 CV (Philips Medical Systems, Best, the Netherlands). The patient was advised to lie in left lateral semirecumbent or supine position during the test, according to the criteria of the American Thoracic Society of Echocardiography (Douglas et al. 2007). Right atrial and ventricle diameters and systolic PAP (sPAP) using echo Doppler estimation of tricuspid regurgitant wave velocity were measured.

Six-minute walk testing

The 6-MWD was conducted according to the current guidelines of the American Thoracic Society (ATS 2002). We used a 30-m long hospital corridor, which was marked by coloured tape at each end. Patients were instructed to walk from end to end, while attempting to cover as much distance as possible in 6 min. During the test, a research assistant timed the walk and recorded the distance reached, the Borg scores for dyspnea as well as heart rate, blood pressure and oxygen saturation. If oxygen supplementation was required the research assistant carried a portable 2.5 kg oxygen tank (Helios™; Marathon, Linde, Germany) with a prescribed oxygen flow to maintain oxygen saturation >90% for the patient.

Cardiopulmonary exercise testing

A symptom-limited exercise test was performed using a cycle-ergometer (ER900 LSE, Oxycon Pro; Viasys

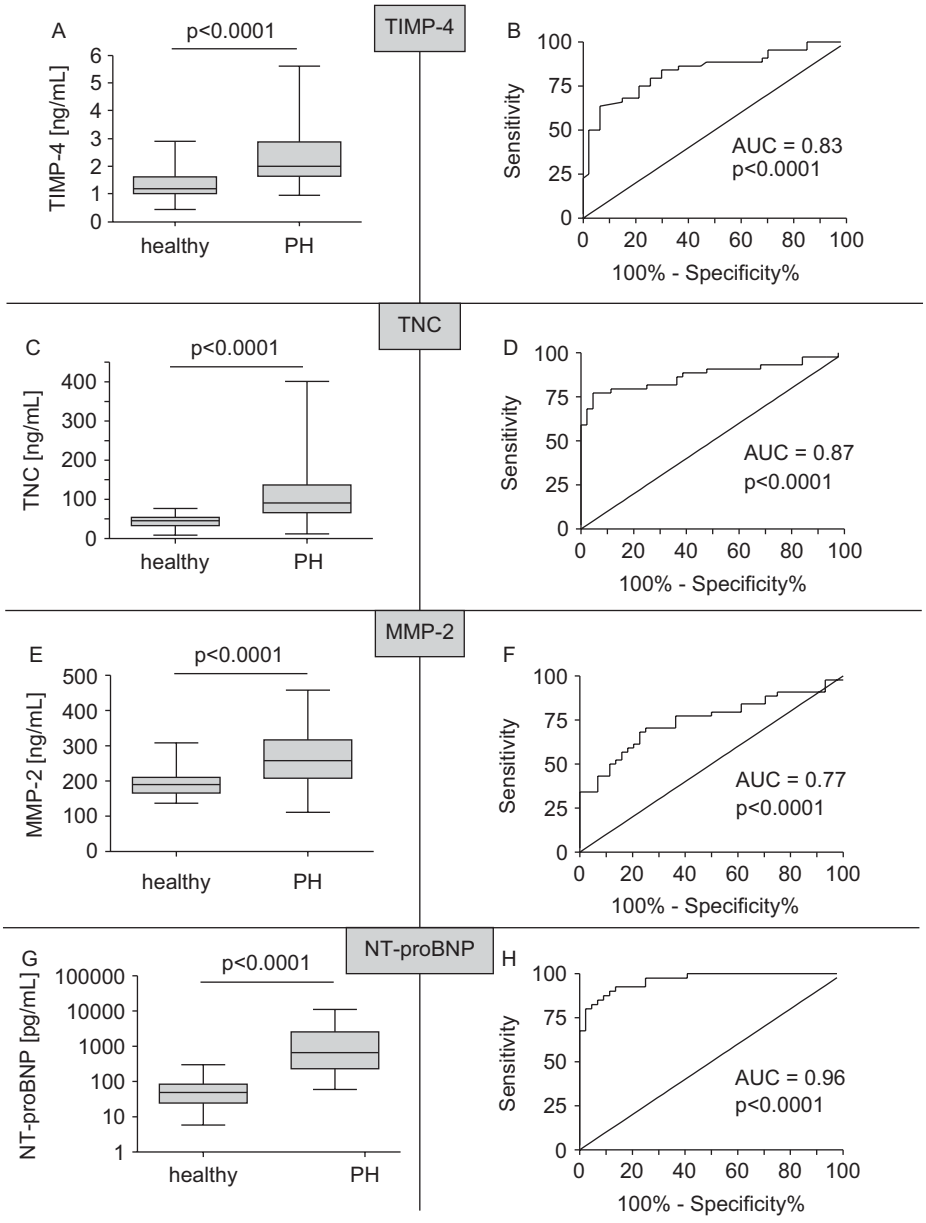

Figure 1. Comparison of plasma biomarker levels. Box plots show plasma biomarker concentrations in pulmonary hypertension (PH) patients ($n = 36$) and healthy controls ($n = 44$): (A) tissue inhibitor of matrix metalloproteinases (TIMP)-4, (C) tenascin C (TNC), (E) matrix metalloproteinase (MMP)-2 and (G) N-terminal b-type natriuretic peptide (NT-proBNP). Data are displayed as median (bar), 25–75th percentile (box) and highest and lowest data point (whiskers). Diagnostic performance of biomarkers was assessed by applying the area under the curve receiver operating characteristic analysis (AUC-ROC). Sensitivity and specificity data for every potential cut-off point for TIMP-4 (B), TNC (D), MMP-2 (F) and NT-proBNP (H) was calculated.

Healthcare, San Diego, CA, USA). Ergospirometry was started at 20 W with a stepwise increment of 10 W min^{-1}. Besides work rate, expired airflow, heart rate and systemic arterial pressure, minute ventilation, expired oxygen and carbon dioxide concentrations, and expiratory carbon dioxide fraction were assessed breath-by-breath. ECG and pulse oximetry were continuously monitored during the test. For calculation of alveolar–arterial oxygen gradient additional assessment of arterial blood gas values were performed at rest and at peak exercise (ABL 555; Radiometer). Peak oxygen uptake was defined as the highest 30-s average of oxygen uptake in the last minute of exercise.

Lung function tests

Pulmonary functions tests were performed before exercise tests using the constant-volume body plethysmograph (MasterScreen; Viasys Healthcare). Airway resistance, residual volume, inspiratory vital capacity,

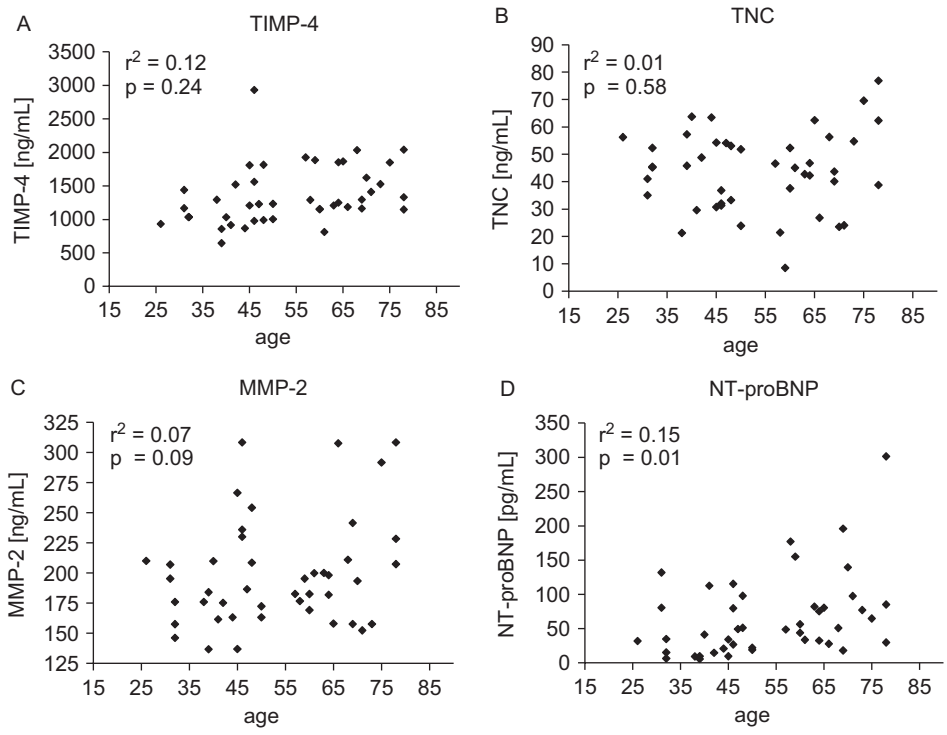

Figure 2. Linear regression analyses. No relationship between TIMP-4, TNC and MMP-2 and age has been found. NT-proBNP tended to be age-dependent. (A) tissue inhibitor of matrix metalloproteinases (TIMP)-4, (B) matrix metalloproteinase (MMP)-2, (C) tenascin C (TNC) and (D) N-terminal b-type natriuretic peptide (NT-proBNP).

forced vital capacity and forced expiratory volume in 1 s were assessed. Assessment of lung transfer factor was measured with carbon monoxide using the single-breath technique. All measurements were done according to the revised guidelines of the American Thoracic Society (ATS) and the European Respiratory Society (ERS) (2005).

Statistics

Statistical significance was estimated by one-way ANOVA (Newman–Keuls post test). A value of $p < 0.05$ was considered to be significant, $p < 0.001$ was considered as highly significant. The diagnostic performance of biomarkers was evaluated by (1) assessing sensitivity and specificity for every detected concentration, (2) construction of receiver-operating characteristic curves (ROC) and (3) calculating the respective area under the curve (ROC-AUC). AUCs of diagnostic tests range from 0.5 (no better than chance) to 1.0 (perfect test). Correlation between plasma biomarker concentrations and functional or morphological parameters were evaluated by linear regression analysis. Biomarker concentrations of female and male individuals were assessed by the Mann–Whitney *U* test for paired samples. GraphPad Prism software, version 4.02 (GraphPad Software, San Diego, CA, USA) was applied for all calculations.

Results

The demographic, functional (lung function, 6-MWD, cardiopulmonary exercise testing, echo Doppler) and haemodynamic data are summarized in Table 1. Cardiopulmonary exercise tests and right heart catheterization were not performed in all patients at the time of biomarker sampling. Most of the study population had known history of PH. During exercises no adverse event occurred. A performance-limiting symptom was shortness of breath. Lung function tests revealed nearly normal lung volumes but, in addition to mild hypoxemia and markedly decreased diffusion capacity, a severity related hypocapnia was found.

Plasma TIMP-4 concentration

Plasma TIMP-4 levels were highly significantly increased in PH patients compared with healthy age- and gender-matched volunteers (Figure 1A) (healthy: 1.35 ± 0.07 ng ml^{-1}, PH 2.32 ± 0.18 ng ml^{-1}, $p < 0.0001$, mean ± SEM). Mean plasma TIMP-4 levels were significantly different between patients with higher NYHA classification (NYHA I-II vs NYHA III, $p < 0.05$) and in patients displaying more severe right ventricular (RV) hypertrophy (no and mild hypertrophy vs moderate and severe hypertrophy, $p < 0.001$) (Figure 3). Interestingly, TIMP-4 concentrations were inversely proportional to

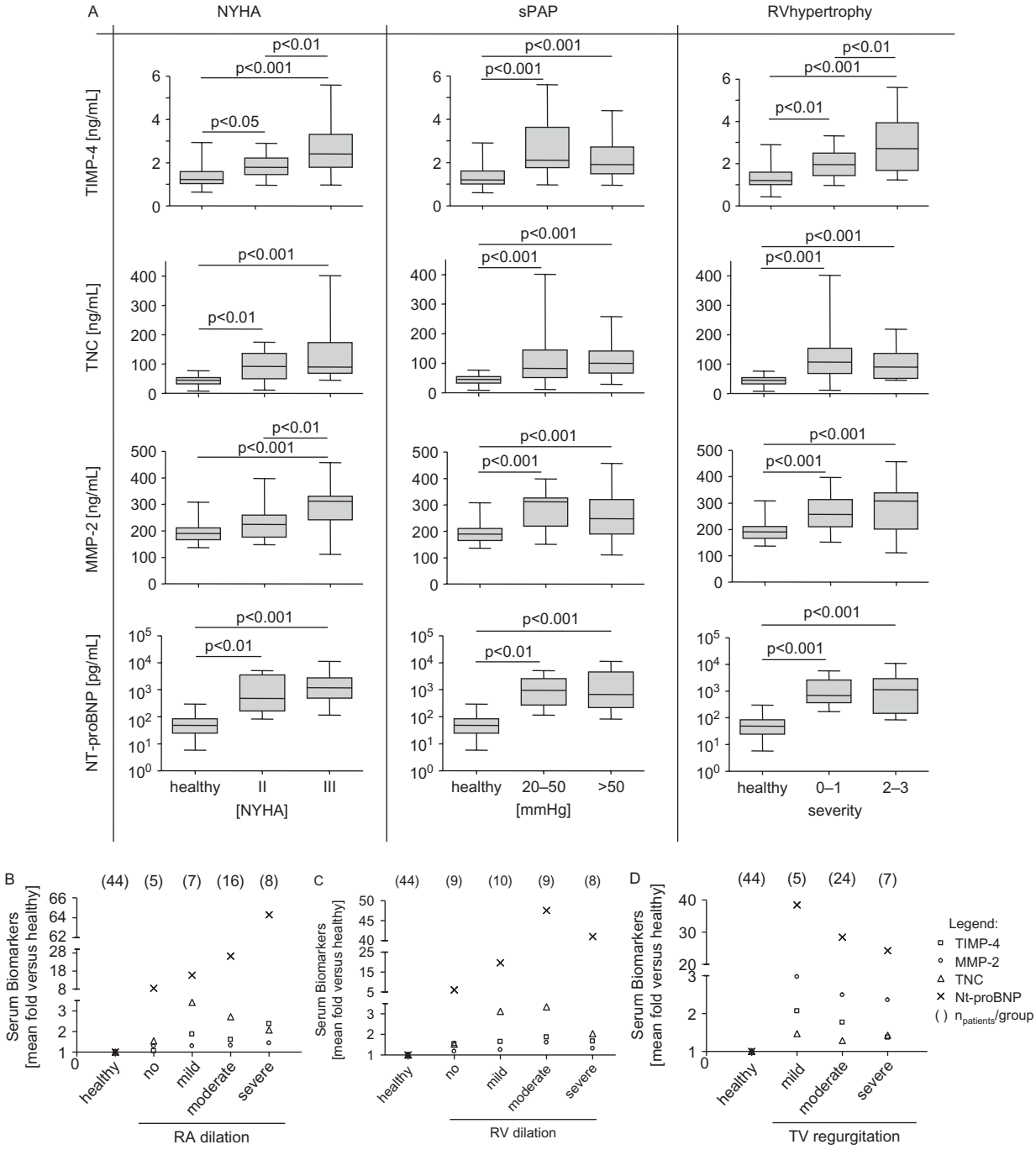

Figure 3. Correlation between disease severity and plasma biomarker concentrations. (A) Box plots show plasma biomarker levels in different patient subpopulations compared with healthy control individuals. Patients were grouped based on disease severity determined as NYHA classification, systolic pulmonary arterial pressure (sPAP) and degree of right ventricular (RV) hypertrophy. In particular, plasma tissue inhibitor of matrix metalloproteinases (TIMP)-4, concentrations discriminate between milder and more severe forms of pulmonary hypertension (PH). Data are displayed as median (bar), 25–75th percentile (box) and highest and lowest data point (whiskers). RV hypertrophy was assessed by echocardiography as described in Materials and methods. RV hypertrophy score: 0 = no; 1 = mild; 2 = moderate; 3 = severe. (B, C) Mean plasma biomarker concentrations of patients with no, mild, moderate or severe right atrial (RA) or RV dilation were normalized against mean serum biomarker concentrations of healthy individuals. (D) Mean plasma biomarker concentrations of patients with mild, moderate or severe tricuspid valve (TV) regurgitation were normalized against mean serum biomarker concentrations of healthy individuals.

severity of TV regurgitation (Figure 3D). Plasma TIMP-4 concentration in healthy volunteers was neither gender (data not shown) nor age dependent (Figure 2A). ROC analysis revealed that PH patients exhibited more increased plasma TIMP-4 concentrations than 83% of the healthy controls (AUC: 0.83, $p < 0.0001$) (Figure 1B).

Plasma TNC concentration

Plasma TNC levels were highly significantly increased in PH patients compared with healthy age-matched volunteers (Figure 1C) (healthy: 43.8 ± 2.2 ng ml^{-1}, PH 111.2 ± 12.7 ng ml^{-1}, $p < 0.0001$, mean ± SEM). Mean plasma TNC levels did not differ significantly between patients displaying higher NYHA classification and more severe RV hypertrophy compared with individuals suffering from milder forms of PH (Figure 3). Plasma TNC concentration in healthy volunteers was neither gender (data not shown) nor age dependent (Figures 2B). ROC analysis revealed that PH patients exhibited more increased plasma TNC concentrations than 87% of the healthy controls (AUC: 0.87, $p < 0.0001$) (Figure 1D).

Plasma MMP-2 concentration

Plasma MMP-2 concentrations were highly significantly increased in PH patients compared with healthy individuals (Figure 1E) (healthy 199.2 ± 6.7 ng ml^{-1}, PH 265.8 ± 12.8 ng ml^{-1}, $p < 0.0001$, mean ± SEM). Mean plasma MMP-2 was found to be significantly different between patients assigned to NYHA I/II and individuals displaying NYHA III status. Interestingly, MMP-2 concentrations were inversely proportional to severity of TV regurgitation (Figure 3D). MMP-2 concentrations were not increased in patients with highly elevated sPAP (>50) and moderate to severe RV hypertrophy compared with patients displaying milder forms of PH (Figure 3). Plasma MMP-2 concentration in healthy volunteers was neither gender (data not shown) nor age dependent (Figures 2C). ROC analysis revealed that plasma MMP-2 exhibited more increased plasma MMP-2 concentrations than 77% of the healthy controls (AUC: 0.77, $p < 0.0001$) (Figure 1F).

Plasma NT-proBNP concentration

Plasma NT-proBNP concentrations were found to be highly significantly elevated in PH patients compared with healthy blood donors (Figure 1G) (healthy: 65.8 ± 9.0 pg ml^{-1}, PH 1888.0 ± 290.1 pg ml^{-1}, $p < 0.0001$, mean ± SEM). Plasma NT-proBNP concentrations could be used to distinguish between mild and strong elevation of sPAP (20–50 mmHg vs < 50 mmHg, $p < 0.001$) and milder and more severe RV hypertrophy (no and mild hypertrophy vs moderate and severe hypertrophy, $p < 0.001$) (Figure 3). Furthermore, plasma NT-proBNP rose progressively with increased severity of right atrial dilation (Figure 3B). Interestingly, NT-proBNP concentrations were inversely proportional to severity of TV regurgitation (Figure 3D). Plasma NT-proBNP concentrations in healthy volunteers tended to be gender ($NT\text{-}proBNP_{male}$ vs $NT\text{-}proBNP_{female}$, $p = 0.02$) and age dependent (Figures 2D). ROC analysis revealed that plasma NT-proBNP can be used to distinguish between PH patients and healthy individuals (AUC: 0.96, $p < 0.0001$) (Figure 1H).

Calculation of cut-off values

The detected biomarker concentrations of all analysed samples were plotted against the respective sensitivity and specificity data (Figure 4). The plasma concentration at which sensitivity equals specificity is displayed in the legend of Figure 4.

Comparison of biomarker concentrations in patients with different etiologies

Based on WHO classification, patients were assigned to two groups (class 1 or class 2–4). Biomarker concentrations were significantly higher in both groups compared with healthy individuals. However, there was no significant difference regarding plasma TIMP-4, TNC, MMP-2 and NT-proBNP concentrations between class 1 PH and class 2–4 PH (Table 2).

Discussion

Structural reshaping of pulmonary vessels and cardiopulmonary tissues is mainly controlled by the MMP–TIMP balance. In areas of vascular remodelling an increased expression and activation of MMPs has been found (Patel et al. 1996). Additionally, the myocardium of heart failure patients is characterized by an increased expression and activation of MMPs, which is accompanied by altered regulation of TIMPs. Based on this evidence, we assume that expression of MMPs and TIMPs can serve as early markers of remodelling (Polyakova et al. 2004). Plasma MMP-2 and TIMP-1 concentrations have been found to be elevated in experimental heart failure in rats and in terminal heart failure patients (Kramer at al. 2008, Milting et al. 2008). MMP-2 expression and activity is increased in lung tissue of monocrotaline rats and reflects response to PAH-oriented treatment (Schermuly et al. 2005). We found significantly increased plasma MMP-2 levels in PH patients compared with healthy control individuals. Additionally, we investigated whether MMP-2 plasma

concentrations can be used to distinguish sufficiently between milder and more severe forms of PH and found that MMP-2 levels are significantly different between patients assigned to NYHA I/II and the individuals displaying NYHA III status. In contrast, MMP-2 concentration was not significantly increased in patients with either highly elevated sPAP (>50 mmHg) or moderate to severe RV hypertrophy compared with patients displaying milder forms of the disease. In general, we did not find any correlation between age or gender and plasma MMP-2.

TIMP-4, also called cardiac inhibitor of metalloproteinases (CIMP), is the most recently cloned member of the TIMP family and has been found to be expressed and secreted predominantly in cardiovascular tissues (Greene et al. 1996). In addition, TIMP-4 mRNA and protein were detected in epithelial and plasma cells in lungs of patients suffering from idiopathic pulmonary fibrosis, whereas this gene is barely expressed in healthy lung tissue (Selman et al. 2000). Based on these observations plasma TIMP-4 is predestined to be used as a biomarker to monitor tissue remodelling processes in PH. Interestingly, it has been found that TIMP-4 plasma levels are reduced in patients suffering from hypertrophic obstructive cardiomyopathy after alcohol septal ablation (Stroud et al. 2005). To the best of our knowledge, this study is the first report showing that TIMP-4 is significantly increased in PH patients and that the increase correlates with disease severity as

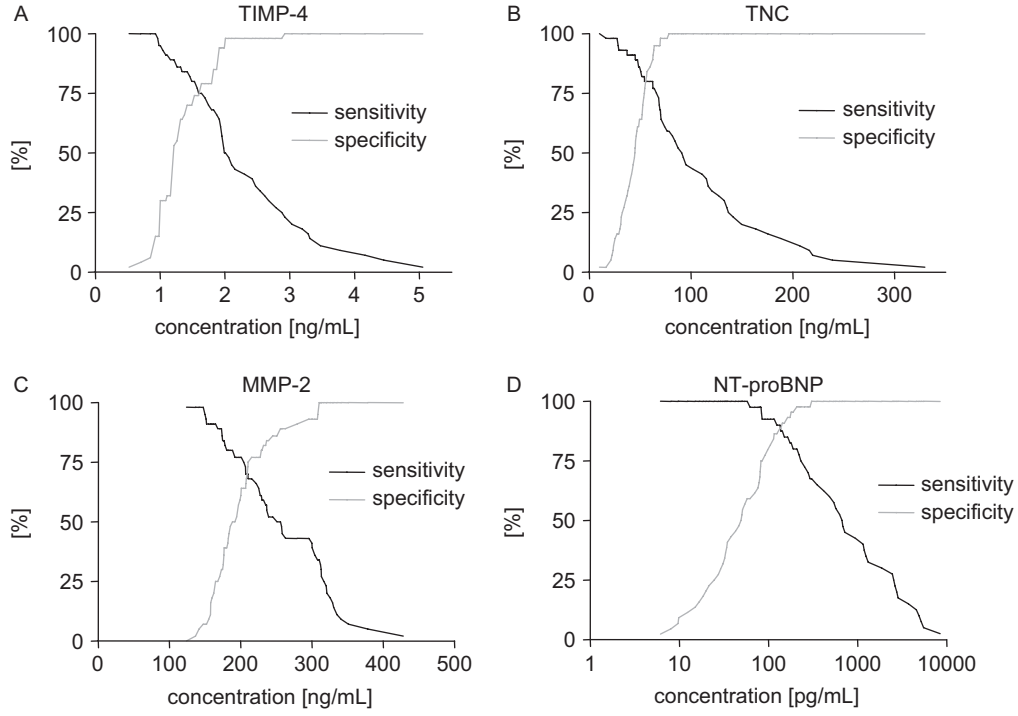

Figure 4. Calculation of cut-off values. The detected biomarker concentrations of all analysed samples were plotted against the respective sensitivity and specificity data. At the intersection of the graphs, sensitivity equals specificity. The corresponding biomarker concentration may serve as a cut-off to discriminate between healthy and pulmonary hypertension (PH). (A) Tissue inhibitor of matrix metalloproteinases (TIMP)-4, (B) tenascin C (TNC), (C) matrix metalloproteinase (MMP)-2 and (D) N-terminal b-type natriuretic peptide (NT-proBNP). Biomarker concentrations at which sensitivity equals or equals approximately specificity are: 1.58 ng ml^{-1} (specificity = 75.0%, sensitivity = 74.5%) for TIMP-4, 55.54 ng ml^{-1} (specificity = sensitivity = 80.0%) for TNC, 209.3 ng ml^{-1} (specificity = sensitivity = 70.0%) for MMP-2 and 137.8 pg ml^{-1} (specificity = 87.5%, sensitivity = 88.6%) for NT-proBNP.

Table 2. Comparison of biomarker concentrations in patients of different etiology.

Biomarker	Healthy	WHO class1	WHO class2–4	WHO class1 vs 2–4
TIMP-4 (ng ml^{-1})	1.35 ± 0.07	2.55 ± 0.26*	2.06 ± 0.24**	n.s.
TNC (ng ml^{-1})	43.82 ± 2.2	125.55 ± 20.41*	94.68 ± 13.62*	n.s.
MMP-2 (ng ml^{-1})	199.2 ± 6.74	274.63 ± 14.77*	255.83 ± 21.6**	n.s.
NT-proBNP (pg ml^{-1})	65.8 ± 9.0	1865.0 ± 460.4*	1945.9 ± 674.4*	n.s.

TIMP, tissue inhibitor of matrix metalloproteinases; TNC, tenascin C; MMP, matrix metalloproteinase; NT-proBNP, N-terminal b-type natriuretic peptide.
*p <0.001 vs healthy; **p <0.01 vs healthy; n.s., not significant.

defined by RV hypertrophy and elevated sPAP. In addition we found that plasma TIMP-4 concentration was neither gender nor age dependent.

TNC is an ECM glycoprotein that is expressed in embryonic development, is upregulated upon tissue injury and is involved in processes of tissue remodelling (Imanaka-Yoshida et al. 2001, Jones & Jones 2000). TNC mRNA and protein expression have been found to be elevated in monocrotaline-induced PAH in rats (Schermuly et al. 2005). Additionally, in cultured pulmonary arteries from monocrotaline-treated rats the elevated expression of TNC can be reduced by direct inhibition of MMP-2, which leads to the induction of apoptosis, loss of ECM and subsequently to regression of pulmonary artery hypertrophy (Cowan et al. 2000). Furthermore, increasing evidence is accumulating that TNC suppresses apoptosis and promotes proliferation of vascular smooth muscle cells (SMCs) (Jones & Jones 2000). In the present report we show that plasma TNC concentrations are significantly increased in PH patients. Plasma TNC concentration was neither gender nor age dependent.

ROC analysis of our data demonstrated that AUC for all assessed remodelling biomarkers were ≥0.77 ($AUC_{TIMP-4} = 0.83$, $AUC_{TNC} = 0.87$, $AUC_{MMP-2} = 0.77$). In comparison to the pressure overload biomarker NT-proBNP, the dynamic range of plasma concentrations of the potential remodelling markers TIMP-4, TNC and MMP-2 is rather small. The strong increase of plasma NT-proBNP concentrations under conditions of increased myocardial stretching made the definition of rule in and rule out cut-off values for the diagnosis of heart failure possible (Collins et al. 2003). For the determination of cut-off values for TIMP-4, TNC and MMP-2 a larger number of patients have to be studied. Interestingly, we found that in patients with severe RV dilation and severe TV regurgitation some biomarkers tended to be decreased compared with patients with moderate dilation and TV regurgitation.

Nevertheless, longitudinal assessment of the new remodelling biomarkers TIMP-4, TNC and MMP-2 in individual patients to monitor response to treatment can be justified based on our observations.

Our study has several potential limitations. First, due to the limited availability of PH patients, only 36 patients were analysed. Second, the cohort of PH patients was very heterogeneous. Patients had different etiologies of PH as well as different treatment strategies at the time of study recruitment. Third, we assessed plasma biomarker concentrations only at a single time point. Longitudinal evaluation of biomarker levels should be performed in future studies.

Nevertheless, the present study demonstrates that plasma concentrations of TIMP-4, TNC and MMP-2 are elevated in PH patients compared with healthy control individuals. In addition we found that a set of biomarkers, which reflect different pathomechanisms of PH, might be useful to (1) assess the status of tissue remodelling and (2) to detect beneficial effects of pharmacotherapy before clinical endpoints are reached in clinical trials, for example. Monitoring of plasma TIMP-4, TNC and MMP-2 levels in PH patients might complement the information which can be gained by the detection of the pressure overload biomarker BNP/NT-proBNP. In particular, plasma concentrations of the remodelling biomarker TIMP-4 mirrored the severity of PH. Neither the remodelling biomarkers TIMP-4, TNC and MMP-2 nor NT-proBNP were statistically significant different between PAH (WHO class 1) and other forms of PH (WHO class 2–4). Certainly, right ventricular hypertrophy and remodelling of the pulmonary vasculature in PH is driven by distinct regulators. Therefore, different plasma biomarkers might help to track the complex underlying pathological processes in this disease.

It remains to be established whether plasma TIMP-4, TNC and MMP-2 concentrations reflect response to treatment in PH patients and therefore can be useful for the guidance of therapeutic decisions in the future.

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Declaration of interest

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