Original Research Article

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Serum cartilage oligomeric matrix protein is decreased in patients with pulmonary hypertension: a potential protective factor

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Abstract

Cartilage oligomeric matrix protein (COMP) was a protective factor in the cardiovascular system. Previous studies showed that hypoxia led to decreased COMP in rat models of pulmonary hypertension. However, the expression pattern of COMP in the pulmonary hypertension population was unclear. A total of 35 patients newly diagnosed with pulmonary hypertension and 70 controls were enrolled in the study. Circulating COMP concentrations of serum samples were measured by enzyme-linked immunosorbent assay and were analyzed the association with multiple clinical variables. Serum COMP concentrations in the pulmonary hypertension group were significantly declined in comparison with age- and sex-matched normal controls, especially in the female subgroup. No significant difference of COMP concentrations was observed in the etiological classification, heart function classification, and risk stratification. Major hemodynamic parameters, six-minute walk distance, N-terminal pro brain natriuretic peptide, and short-term prognosis were not statistically associated with COMP. However, some echocardiography parameters, like tricuspid annular plane systolic excursion and mean right atrial pressure, were found the negative relation to COMP concentrations. In conclusion, serum COMP levels were decreased in the patients with pulmonary hypertension, which was in accordance with its known biological effects. Its association with long-term prognosis was worth further exploring.

Keywords

cartilage oligomeric matrix protein (COMP), pulmonary hypertension (PH), tricuspid annular plane systolic excursion (TAPSE)

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Introduction

Pulmonary hypertension (PH) is defined as mean pulmonary arterial pressure (mPAP) ≥25 mmHg, with the characteristics of obstructive remodeling of pulmonary small arteriole and progressive right heart failure.[1] Despite the emerging targeted drug therapy is effective, the prognosis of patients with PH is still poor, especially for those at high-risk stratification whose one-year mortality rate is as high as 20%.[2] Thus, it is necessary to explore undiscovered pathogenic mechanisms in PH.

Cartilage oligomeric matrix protein (COMP), also named as thrombospondin-5, is a glycoprotein which could interact with collagens, fibronectin, and integrins.[3] It was first identified in articular cartilage, and its deficiency was associated with pseudoachondroplasia and multiple epiphyseal dysplasia.[3] In the cardiovascular system, COMP was expressed in platelets, vascular smooth muscle cells (VSMCs), and cardiomyocytes, playing a protective role and negatively regulating thrombosis.[4,5]

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COMP could block the signaling of bone morphogenetic protein (BMP)-2 in VSMCs to reduce vascular calcification.[6] Conversely, knockdown of COMP in pulmonary artery smooth muscle cells (PASMCs) resulted in downregulation of BMP-2 and BMP receptor 2.[7] Furthermore, the deficiency of COMP also resulted in enhanced proliferation, increased oxidative stress, and decreased contractile markers of PASMCs.[7,8] As for animal models of PH, chronic hypoxia of three weeks led to decreased COMP in the pulmonary vasculature of rat models and mice models.[7,8]

We argued that COMP might be a promising biomarker or target, but its distribution in the PH population was still unclear. In this study, we aimed to explore the expression pattern of COMP in the PH population and analyzed its association with multiple clinical variables.

Method

Participant recruitment

From December 2017 to June 2019, the hospitalized patients newly diagnosed with PH were recruited. The diagnosis criteria of PH were based on the 2015 European Society of Cardiology and European Respiratory Society Guidelines: right heart catherization-determined mPAP $\geq$25 mmHg at rest and at sea level.[1] Some age- and sex-matched normal controls were also recruited into this study with the PH-to-control ratio of 1:2.

Collection of medical information and serum sample and follow-up

In the control group, only age and sex were recorded. In the PH group, information on underlying diseases, blood routine examination, blood biochemistry tests, electrocardiogram, echocardiography, right cardiac catheterization, and six-minute walk distance was collected. Information on initial therapies was also recorded, including surgery, transcatheter closure of defects, targeted therapeutics, anticoagulant therapy, and other cardiovascular medications. Baseline blood samples were collected before the initial treatment using serum separator tubes. They were centrifuged at 3000 r/min for 15 min and stored at -80°C in single-dose vials.

Patients were required for a reexamination of echocardiography or N-terminal pro brain natriuretic peptide (NT-proBNP) after discharge. We classified the short-term prognosis into three grades of "improvement, stability and progression," according to the results of echocardiography and NT-proBNP. "Improvement" was defined as declined systolic PAP by more than 10mmHg or declined NT-proBNP by more than twofold. "Progression" was defined as increased systolic PAP by more than 10mmHg or increased NT-proBNP by more than twofold. Other conditions were categorized into "stability." Patients who did not come back for review were contact via telephone to get their survival information. The last time of follow-up was March 2021.

Enzyme-linked immunosorbent assay

Commercial COMP enzyme-linked immunosorbent assay kit (the catalog number: EK0913) was purchased from BOSTER Biological Technology Co. Ltd (Wuhan, China). The intra-assay coefficient of variation (CV%) ranged from 4.2% to 6.8%, and inter-assay CV% was from 4.9% to 7.3%. Experiment procedure was performed according to the instruction. The suitable dilution ratio of serum was determined as 50 fold by a preliminary experiment. A linear regression was analyzed to fit the standard curve and calculate the serum concentrations.

Statistical analysis

All the continuous variables were expressed as the median and interquartile range (IQR) and analyzed by Mann–Whitney test for two groups' comparison. One-way analysis of variance test was used to make comparison among multiple groups and Dunnett test was used to make pairwise comparisons. The categorical variables, like sex ratio, were analyzed by Chi-square test. Univariate linear regression analysis was used to calculate the correlation between serum COMP concentration and many clinical variables. Independence and normality of residuals were judged by Durbin–Watson method, histogram, and normal probability plot. IBM SPSS statistics 23 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6 (GraphPad Software, CA, USA) were used to perform the above statistical analysis. The receiver operating characteristic (ROC) curve was performed by MedCalc software v19.5.3 (MedCalc Software Ltd, Ostend, Belgium). A two-tailed $P < 0.05$ was regarded as statistical significance.

Result

All the normal subjects (n = 70) in the control group were between 35 and 40 years old, and the sex ratio of male to female was 2:5. Similarly, the median and IQR of age in the PH group (n=35) was 33 (30–36), and the sex ratio was 1:5. Table 1 shows the baseline characteristics of patients with PH in three etiological subgroups. The results of echocardiography and right heart catherization demonstrated that patients with PH had increased PAP and resistance as well as progressive right heart failure (Table 1). Additionally, their variables of systematic circulation were in the relatively normal range.

In general, the serum concentrations of COMP in the PH group (420.1 ± 14.25 ng/ml) were significantly lower than that in the control group (468.9 ± 6.53, ng/ml, $P<0.001$, Fig. 1a). Moreover, the area under the ROC was 0.713 ($P<0.001$) (Fig. 1b). At the cut-off value of 411.5 ng/ml, the sensitivity was 54.3% and the specificity was 88.6%.

Table 1. Basic characteristics of patients with PH.

Parameter	IPAH (n = 11)	CHD-PH (n = 17)	Other (n = 7)
Age (years)	35 (30–39)	31 (29–34)	35 (29–36)
Female (n, %)	9 (81.8)	14 (82.4)	6 (85.7)
WHO functional class (n, %)			
I–II	2 (18.2)	2 (11.8)	2 (28.6)
III–IV	9 (81.8)	15 (88.2)	5 (71.4)
Six-minute walk distance (m)	420 (385–463)	380 (340–470)	394 (363–426)
Venous oxygen saturation (%)	71.5 (64.0–74.0)	59.3 (56.3–66.5)	65.8 (63.0–76.0)
Cardiac output (L/min)	3.8 (3.0–5.9)	3.0 (2.7–5.2)	3.5 (3.2–4.4)
NT-proBNP (ng/ml)	1470 (554–2339)	625 (186–1406)	1839 (527–3456)
Pulmonary artery diameter (mm)	28 (24–29)	36 (29–41)	30 (26–31)
TAPSE (mm)	16 (12–19)	17 (14–18)	13 (11–16)
mPAP (mmHg)	58 (47–75)	63 (55–90)	43 (37–50)
mRAP (mmHg)	4 (3–11)	3 (1–8)	3.5 (2–8)
mRVP (mmHg)	11 (4–16)	8 (5–11)	6 (2–12)
PCWP (mmHg)	4.0 (2.0–5.3)	0 (0–6.0)	3.0 (1.0–4.5)
Total pulmonary resistance (Wood units)	10.5 (7.2–19.7)	15.6 (9.5–21.3)	11.0 (7.1–15.5)
Heart rate (beats/min)	82 (78–98)	78 (72–85)	85 (78–100)
Systolic blood pressure (mmHg)	120 (116–131)	115 (107–120)	118 (98–126)
Diastolic blood pressure (mmHg)	80 (70–89)	70 (65–77)	77 (58–90)

Note: All the numerical data were shown as median (interquartile range), and all the binary data were shown as number (percentage). The other group consisted of five cases with connective tissue disease-associated PH and two cases with chronic thromboembolic PH. PH: pulmonary hypertension; WHO: World Health Organization; CHD: congenital heart disease; IPAH: idiopathic pulmonary artery hypertension; NT-proBNP: N-terminal pro brain natriuretic peptide; TAPSE: tricuspid annular plane systolic excursion; mPAP: mean pulmonary artery pressure; mRAP: mean right atrial pressure; mRVP: mean right ventricular pressure; PCWP: pulmonary capillary wedge pressure.

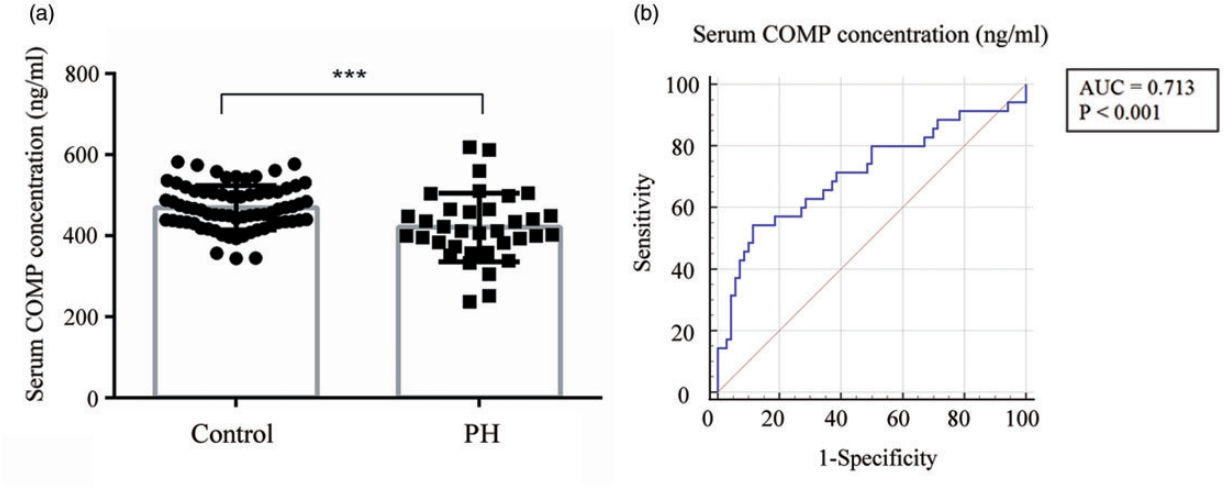

Fig. 1. Serum COMP concentrations in patients with PH (n = 35) were significantly reduced in comparison with normal control population (n = 70). COMP: cartilage oligomeric matrix protein; PH: pulmonary hypertension; AUC: area under the ROC curve. ***$P < 0.001$.

Interestingly, we discovered that the phenomenon of decreased serum COMP concentration was obvious in the female and congenital heart disease (CHD) subgroup but not in the male and other etiological subgroups (Fig. 2a and b). Nevertheless, no statistical significance of COMP concentrations was observed in the heart function classification and risk stratification (Fig. 2c and d).

In the linear correlation analysis, we found that the serum COMP concentrations of patients with PH were negatively correlated with tricuspid annular plane systolic excursion (TAPSE, $R^2 = 0.526$, $P = 0.001$) and positively correlated with mean right atrial pressure (mRAP, $R^2 = 0.376$, $P = 0.034$), suggesting that it might be involved in the pathogenic process of right heart failure (Table 2). Fig. 3 further confirmed the negative correlation between TAPSE and COMP in the three etiological subgroups, with the three different colored dots evenly distributing along the trend line. However, other major clinical parameters were

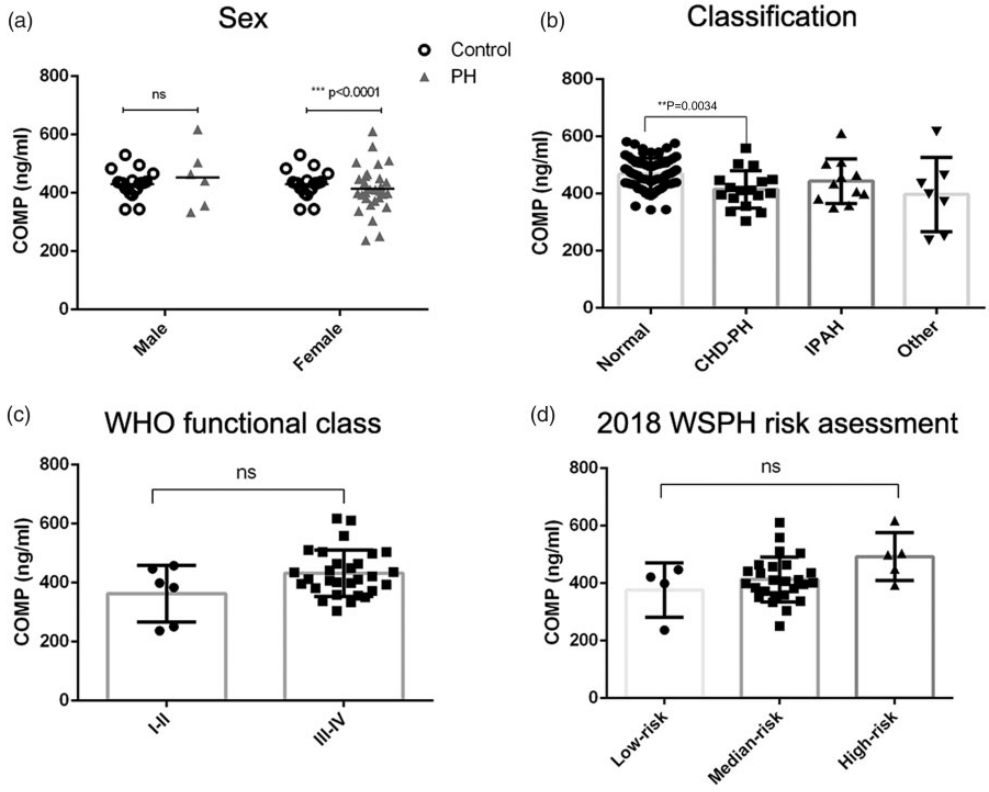

Fig. 2. Serum COMP concentrations were significantly declined in the female and CHD subgroup. The other subgroup was composed of two CTEPH cases and five CTD-PH cases. COMP: cartilage oligomeric matrix protein; PH: pulmonary hypertension; CHD: congenital heart disease; IPAH: idiopathic pulmonary artery hypertension; CTD: connective tissue disease; CTEPH: chronic thromboembolic pulmonary hypertension; WHO: World Health Organization; WSPH: World Symposium on Pulmonary Hypertension; ns: not significant.

Table 2. Linear correlation analysis between COMP and clinical parameters.

Parameter	Beta index / R^2	*P*-value
Age	0.139	0.425
6MWD	−0.177	0.318
SvO_2	−0.146	0.418
Cardiac output	−0.263	0.250
NT-proBNP	0.216	0.213
Cardiac troponin T	0.120	0.491
Pulmonary artery diameter	0.168	0.336
TAPSE	−0.526	0.001
LVEDD	−0.127	0.469
Ejection faction	−0.078	0.655
Mean PAP	0.297	0.088
Systolic PAP	0.320	0.065
Diastolic PAP	0.246	0.162
PVR	0.312	0.208
mRAP	0.376	0.034
mRVP	−0.190	0.298
PCWP	−0.105	0.670

Note: COMP: cartilage oligomeric matrix protein; 6MWD: six-minute walk distance; SvO_2: venous oxygen saturation; NT-proBNP: N-terminal pro brain natriuretic peptide; TAPSE: tricuspid annular plane systolic excursion; LVEDD: left ventricular end-diastolic dimension; PAP: pulmonary artery pressure; PVR: total pulmonary resistance; mRAP: mean right atrial pressure; mRVP: mean right ventricular pressure; PCWP: pulmonary capillary wedge pressure.

not statistically associated with COMP and did not differ between the low- and high-level groups of COMP. We also performed the correlation analysis separately in the three etiological groups. NT-proBNP ($R^2 = 0.789$, $P = 0.035$) and cardiac troponin T ($R^2 = 0.836$, $P = 0.019$) were positively correlated with COMP in the other subgroup, and mRAP ($R^2 = 0.689$, P=0.019) was only positively associated with COMP in the idiopathic pulmonary artery hypertension (IPAH) subgroup.

During the follow-up period, only one patient with chronic thromboembolic PH died. In addition, 63% of patients (n = 22) had a reexamination of echocardiography at our hospital after initial treatment. However, no statistical difference in baseline COMP concentrations was observed based on the echocardiography-determined short-term prognosis ($P = 0.44$).

Discussion

In this study, we demonstrated that the serum concentrations of COMP in the PH group were significantly decreased in comparison with age- and sex-matched normal controls. No statistical significance of COMP concentrations was observed in several classifications and in association with many clinical parameters.

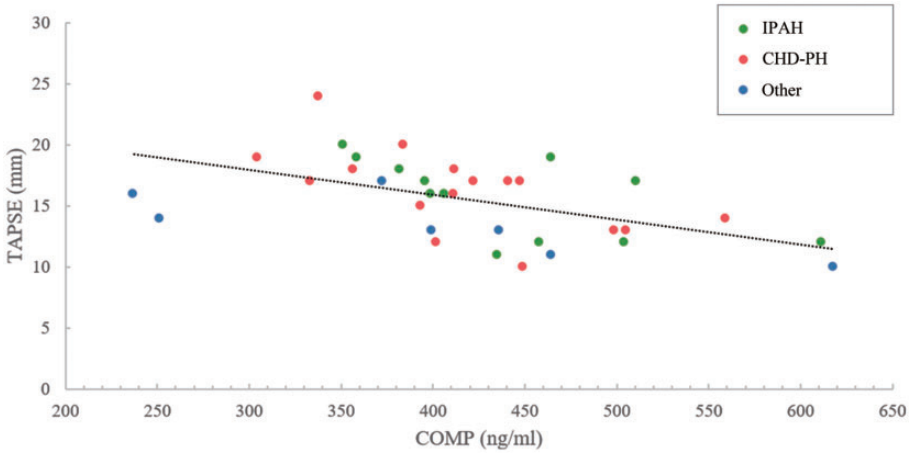

Fig. 3. TAPSE was negatively correlated with serum COMP concentrations in the three etiological subgroups. TAPSE: tricuspid annular plane systolic excursion; COMP: cartilage oligomeric matrix protein; CHD: congenital heart disease; IPAH: idiopathic pulmonary artery hypertension; PH: pulmonary hypertension.

We observed that COMP was downregulated in the serum of PH patients. In PASMC, COMP deficiency led to increased production of reactive oxygen species, and COMP supplement could reverse the adverse effects of hypoxia, suggesting that COMP might participate in the pathogenic process of PH and play a protective role.[8] It was in accordance with our observation of decreased COMP in patients with PH.

Dysregulation of serum COMP was also reported in several diseases, including osteoarthritis, hepatoma, and metastatic breast cancer.[9–11] In idiopathic pulmonary fibrosis, increased concentrations of COMP indicated the accelerated decline of forced vital capacity.[12] From the views of pathological mechanisms, COMP could interact with transforming growth factor beta1 to enhance its activity and promote the fibrotic process.[3,13] In contrary to PH, serum concentrations of COMP were upregulated in the patients with coronary heart disease or with maintenance hemodialysis.[14,15] Moreover, COMP was positively associated with the degree of vascular calcification in the above two study.[14,15] However, COMP played an anticoagulant role and could inhibit the calcification process of VSMCs.[3,6] Therefore, the upregulated COMP in patients with coronary heart disease might be a compensatory mechanism. An experimental study showed that pulmonary arteries and coronary arteries responded differently to hypoxia (3% oxygen) in the aspects of COMP secretion, possibly accounting for the reason why COMP was decreased in PH but increased in coronary heart disease.[8]

In the analysis of relationship between COMP and clinical parameters, we did not observe the decline of COMP in the male subgroup and the IPAH subgroup, which might be due to limited numbers of male and IPAH patients. In addition, a majority of variables and short-term prognosis had no statistical correlation with COMP, which might also result from limited sample size and short follow-up period.

Intriguingly, the positive correlation of COMP concentrations and TAPSE was identified, which was seemingly in contrast to the decline of COMP in the whole PH participants. In fact, the pathogenesis and deterioration of PH are much complicated, and some contradictory results about biomarker studies were reported. For example, endostatin and hepatoma-derived growth factor, which played the opposite roles in angiogenesis, were both increased in the serum of PH patients and correlated with the disease severity.[16–18] Moreover, a previous study showed that COMP deficiency resulted in spontaneous dilated cardiomyopathy in mice.[18] COMP, which could interact with integrin $\beta 1$ and stabilize its expression, promoted the connection between cardiomyocytes and extracellular matrix and increased the mechanical strength of myocardium. Thus, we postulated that the involvement of right heart hypertrophy in the decompensated period of PH might contribute to the upregulation of COMP.

Two limitations should be considered in interpreting our results. The limited sample size might lead to false negative results, such as no difference of COMP concentrations in several different classifications. Besides, the three-year follow-up duration was relatively short, and the outcome of death was few. We could not analyze the association of serum COMP concentrations and mortality. In the future, we plan to confirm our results in a large cohort, further analyze the prediction efficacy of COMP on death outcomes, and explore its protective mechanisms in PH.

In conclusion, serum concentrations of COMP were decreased in the patients with PH, which was in accordance with its known biological effects. Its associations with prognosis should be further explored in a large cohort.

Author contributions

Data collection: Dan-dan Chen, Liang Xie, Gui-ling Xiang, and Qin-han Wu; patient management: Dan-dan Chen, Shan-qun Li, and Li-hua Guan; study design and statistical analysis: Wei-ping

Hu and Dong Liu; manuscript writing: Wei-ping Hu; critical manuscript revision: Jie-ming Qu, Li-hua Guan, and Dong Liu. All authors read and approved the final manuscript.

Consent to participate

All the participants gave the informed consent for the collection of data and samples.

Ethical approval

The study was approved by the Ethical Review Board of Shanghai Zhongshan Hospital. (Approval number: B2018-184R).

Conflict of interest

The author(s) declare that there is no conflict of interest.

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