


# Increased Levels of Runt-Related Transcription Factor 2 Are Associated With Poor Survival of Patients With Idiopathic Pulmonary Arterial Hypertension

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## Abstract

Runt-related transcription factor 2 (RUNX2) plays a pivotal role in the pathogenesis of pulmonary arterial hypertension (PAH); yet, whether circulating levels of RUNX2 can predict survival of patients with idiopathic PAH (IPAH) is still unclear. The present study aimed to investigate the correlation between circulating levels of RUNX2 and survival of patients with IPAH. Blood samples were collected from 46 incident patients with IPAH and 30 healthy controls in Shanghai Pulmonary Hospital. Levels of RUNX2 were measured using ELISA. Linear regression and cox proportional hazards analysis were performed to assess the prognostic value of RUNX2 levels in predicting survival using the Kaplan–Meier method. Nonsurvivors had significantly shorter 6MWD, higher levels of NT-proBNP, increased mRAP, mPAP, mPAWP, PVR, and decreased CO as well as CI, compared with survivors ( $p < .05$ ). Plasma levels of RUNX2 were significantly higher in nonsurvival and survival patients with IPAH compared with controls ( $p \leq .001$ ), and higher in nonsurvivors than in survivors ( $p = .001$ ). RUNX2 levels served as an independent predictor of survival in these patients ( $p < .001$ ). RUNX2 levels  $\geq 41.5$  ng/ml had a sensitivity of 80.0% and a specificity of 74.2% by ROC analysis. Patients with a RUNX2 level  $< 41.5$  ng/ml and/or mRAP  $< 3.5$  mmHg had a significantly better prognosis than those with a higher RUNX2 level in all subjects as well as in male or female patients ( $p < .05$ ). The level of circulating RUNX2 is an independent predictor for survival and it is correlated with the clinical severity of IPAH.

## Keywords

idiopathic pulmonary arterial hypertension, RUNX2, survival, sex difference

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Idiopathic pulmonary arterial hypertension (IPAH), a complex condition with unknown etiology, remains a progressive disease leading to premature death despite the availability of effective targeted therapies (Galiè et al., 2013; Humbert et al., 2006; Jing et al., 2007). Its prognosis is poor with a 5-year survival rate of 50% (Benza et al., 2012; Manes et al., 2014). One of the major pathophysiological characteristics is the hyperproliferative and apoptosis-resistant smooth muscle cells of the pulmonary artery contributing to pulmonary artery remodeling. A variety of pathological conditions such as mitochondria-endoplasmic reticulum stress (Sutendra

et al., 2011), activation of the receptor of advanced glycation end-products (Meloche et al., 2014), DNA damage (Federici et al., 2015), and abnormal expression of microRNAs(miRNAs). (Courboulin et al., 2011), could aggravate pulmonary vascular calcification, leading to pulmonary arterial hypertension (PAH). Among these pathological conditions, abnormal expression of miRNAs is associated with increased levels of runt-related transcription factor 2 (RUNX2).

RUNX2 is a transcription factor belonging to the RUNX family which comprises three members: RUNX1, RUNX2, and RUNX3. Despite their structural



similarities, RUNX proteins have divergent physiological roles. RUNX1 and RUNX3 are essential for definitive hematopoiesis and gastrointestinal and neuronal development. RUNX2 is important for the differentiation of osteoblasts and chondrocytes (Komori, 2010; Ruffenach et al., 2016). It can drive mesenchymal stem cells to differentiate into the osteoblastic lineage which is responsible for bone mineralization (Komori, 2010). In addition, RUNX2 activates a number of osteogenic components, such as type 1 collagen, osteopontin, and osteocalcin (Schroeder et al., 2005). The level of RUNX2 is very low in normal vascular cells, but significantly elevated in remodeled or calcified vascular tissue. This suggests that RUNX2 might be an important player in the vascular pathology of PAH. Indeed, one study supports the concept that vascular calcification is an active cell-driven process characterized by the acquisition of osteogenic phenotype of vascular cells (Schroeder et al., 2005).

Ruffenach et al. (2016) have reported that RUNX2 is upregulated in lungs, distal pulmonary arteries, and primary pulmonary artery smooth muscle cells (PASCs) isolated from PAH patients compared to PASCs isolated from patients without PAH. This suggests that RUNX2 plays a pivotal role in the pathogenesis of PAH by contributing to the proliferation and calcification of the pulmonary artery (Ruffenach et al., 2016). However, there is little research on the potential link between the level of plasma RUNX2 and the prognosis of patients with IPAH. The present study aimed to answer this question by measuring levels of circulating RUNX2 and correlating them with clinical outcomes of patients with IPAH.

## Method

### Study Participants

Forty-six (18 males) incident patients with IPAH and 30 age- and gender-matched healthy controls (12 males) aged 18 or older were recruited from the Department of Cardio-Pulmonary Circulation, Shanghai Pulmonary

Hospital, between May 2012 and July 2018. Diagnosis of IPAH was based on the European Society of Cardiology/European Respiratory Society criteria (Galiè et al., 2016). Patients with PAH due to a definite cause such as connective tissue diseases, congenital heart diseases, portal hypertension, chronic pulmonary obstruction, chronic pulmonary thromboembolism, or pulmonary hypertension due to left heart diseases were excluded. Patients with acute or chronic illnesses that might influence hormonal metabolism (i.e., acute or chronic infections, chronic autoimmune diseases, previously established primary endocrine disorders) and patients who were treated with hormones (thyroid hormones, anabolic steroids, corticosteroids) or drugs that markedly inhibit hormone production, either at the time of the study or in the past, were also excluded. The study procedure was approved by the ethics committee of Shanghai Pulmonary Hospital (Study#K08-015C). Written informed consent was obtained from all participants.

### Clinical Assessment

Demographic information including age, gender, body mass index (BMI), concomitant conditions, 6-minute walk distance (6MWD) test, World Health Organization functional classification (WHO FC), NT-proBNP, and hemodynamics were determined at admission. Right heart catheterization (RHC) was performed as described previously (Jing et al., 2009). The 6MWD test was performed according to the American Thoracic Society (ATS) guideline, and the Borg dyspnea score was determined immediately after completing the 6MWD test (American Thoracic Society, 2002).

Fasting ethylenediamine tetraacetic acid (EDTA) anticoagulated blood samples were collected for initial assessment. Plasma was immediately isolated and frozen at  $-20^{\circ}\text{C}$ . It was stored at  $-80^{\circ}\text{C}$  until all samples were ready for measurement. Concentrations of plasma RUNX2 were tested using the enzyme-linked immunosorbent assay in the biochemistry laboratory of Shanghai Pulmonary Hospital.

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**Table 1.** Baseline Characteristics of Survivors and Nonsurvivors.

	Nonsurvivors (n = 15)	Survivors (n = 31)	p value
<b>Baseline characteristics</b>			
Age, y	38.5 ± 15.5	47.5 ± 15.0	.568
Male/female, N	6/9	12/19	.935
HR, bpm	86.6 ± 10.5	78.0 ± 16.5	.025
SBP, mm Hg	115.2 ± 20.6	115.7 ± 20.0	.938
DBP, mm Hg	70.7 ± 13.1	71.0 ± 14.5	.961
BMI, kg/m <sup>2</sup>	23.2 ± 3.6	21.7 ± 2.6	.129
6MWD, m	323.0 ± 89.8	382.1 ± 88.9	.041
NT-proBNP	400.0 (310.0, 450.0)	305.0 (275.5, 377.5)	.035
WHO-FC, n (%)			.332
I–II	6 (40.0)	15 (48.4)	
III–IV	9 (60.0)	16 (51.6)	
<b>Hemodynamics</b>			
mRAP, mm Hg	6.9 ± 5.1	3.6 ± 3.5	.013
mPAP, mm Hg	59.3 ± 11.3	50.0 ± 8.7	.003
mPAWP, mm Hg	8.7 ± 4.2	6.5 ± 2.7	.035
PVR, Wood units	13.6 ± 4.8	10.2 ± 3.5	.009
CO, L/min	3.9 ± 1.1	4.7 ± 1.2	.040
CI, L/min/m <sup>2</sup>	2.5 ± 0.7	2.9 ± 0.6	.037
<b>Specific medications</b>			
PDE-5 inhibitors, %	9 (50.0)	11 (16.7)	.096
ERAs, %	4 (25.0)	6 (16.7)	
Prostacyclin analogs, %	0 (25.0)	1 (8.3)	
Combination, %	2 (0.0)	8 (16.7)	
Nonspecific medication, %	0 (0.0)	5 (8.3)	

Note. 6MWD = 6-minute walk distance, BMI = body mass index, CI = cardiac index, CO = cardiac output, ERA = endothelial receptor antagonist, HR = heart rate, mPAP = mean pulmonary arterial pressure, mPAWP = mean pulmonary capillary wedge pressure, mRAP = mean right atrial pressure, PDE-5 = phosphodiesterase type 5, PVR = pulmonary vascular resistance; RUNX2 = runt-related transcription factor 2, WHO-FC = World Health Organization functional classification.

### Statistical Analysis

All data were expressed as mean ± SD or medians (and interquartile range) for continuous variables and as the absolute number for categorical variables. Comparisons were completed using either *t*-test or the Mann–Whitney U test for continuous variables and the  $\chi^2$  test for categorical variables. Correlations were assessed using the univariate and multivariate linear regression analysis. Univariate and multivariate linear regressions and the cox proportional hazards analysis were performed using baseline levels of RUNX2 in combination with hemodynamic variables to assess the prognostic value of each of them for predicting survival. Using a forward stepwise multivariate model, the prognostic power of RUNX2 was compared with that of other significant parameters found in univariate analysis. The multivariate analysis model was adjusted for age, sex, and BMI. Receiver-operating characteristic (ROC) curves for independent parameters were drawn, and the areas under the curves were calculated. The cut-off value, sensitivity, and specificity of independent parameters changed in nonsurvivor and

survivor were measured by an ROC analysis. Survival was estimated using the Kaplan–Meier method and analyzed with the log-rank test after grouping patients using the cut-off value of each independent predictor. A *p* value <.05 was considered significant. Statistical analysis was performed using SPSS (Statistic Package for Social Science, Chicago, IL) version 26.0.

## Results

### Characteristics of Study Participants

A total of 46 patients with IPAH met the inclusion criteria and 30 healthy controls were recruited to the present study. Among patients with IPAH, 28 (61%) were female and 18 (39%) were male. The mean duration of follow-up was 34.7 ± 15.8 months. Six (13%) male and nine (20%) female patients died during this period. None of the patients were lost for follow-up, resulting in a 100% follow-up rate. The average ages were 38.5 ± 15.5 for nonsurvival and 47.5 ± 15.0 years for survival patients. Demographic and hemodynamic data are presented in Table 1.

There were no differences in age, BMI, blood pressure (BP), and WHO FC between nonsurvivors and survivors. Nonsurvivors had significantly lower levels of 6MWD and higher levels of NT-proBNP than survivors ( $p = .041$  and  $p = .035$ ; Table 1). Nonsurvivors had significantly higher values of hemodynamic parameters including mean right atrial pressure (mRAP), mean pulmonary arterial pressure (mPAP), mean pulmonary capillary wedge pressure (mPAWP), and pulmonary vascular resistance (PVR) at diagnosis than survivors, in contrast to their cardiac output (CO) and cardiac index (CI) values ( $p = .013$ ,  $p = .003$ ,  $p = .035$ ,  $p = .009$ ,  $p = .040$ , and  $p = .037$ ; Table 1).

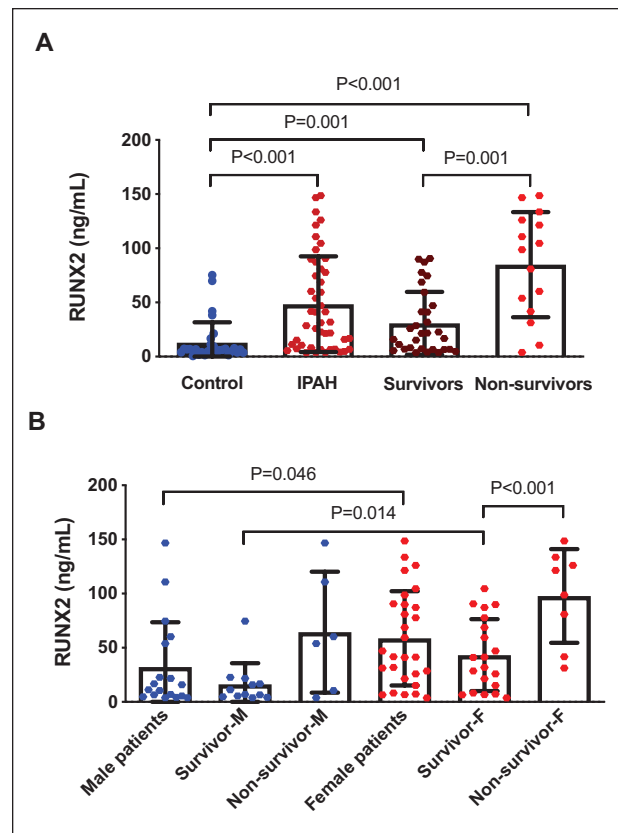
Targeted PAH medications included inhaled iloprost, intravenous iloprost, oral beraprost, oral bosentan, oral ambrisentan, oral sildenafil, oral vardenafil, and oral tadalafil. There were no significant differences in the intake of these medications between nonsurvivors and survivors (Table 1).

### Expression of RUNX2 in Survivors and Nonsurvivors With IPAH

All patients with IPAH had significantly higher RUNX2 levels than controls (patients: 31.5 [8.0, 82.5] vs. controls: 6.6 [3.9, 9.9],  $p < .001$ ; Figure 1A). For the analyses of the subgroups, RUNX2 levels were significantly increased in survivors as well as in nonsurvivors with IPAH compared to controls ( $p = .001$  and  $p < .001$ ; Figure 1A). Survivors had significantly lower RUNX2 levels than nonsurvivors ( $p = .001$ ; Figure 1A). There was a significant sex difference in RUNX2 levels between male and female patients (Figure 1B). Female patients had significantly higher RUNX2 levels than male patients ( $p = .046$ ; Figure 1B). This also applies to female and male survivors ( $p = .014$ ; Figure 1B). Female survivors had significantly lower RUNX2 levels than female nonsurvivors ( $p < .001$ ; Figure 1B). A similar trend was observed in male patients, but no significant difference was found between male survivors and male nonsurvivors (Figure 1B).

### Correlations Between Levels of Plasma RUNX2 and Hemodynamic Parameters

A positive correlation was observed between plasma RUNX2 levels and mPAP as well as PVR ( $p = .028$  and  $p = .001$ ; Table 2), whereas a negative correlation was found between plasma RUNX2 levels and CO ( $p = .045$ ; Table 2). No significant correlation was found between plasma RUNX2 levels and mRAP, mPAWP, or CI. After adjusting age, sex, and BMI, plasma RUNX2 levels were included in the stepwise multiple regression analyses to



**Figure 1.** Levels of RUNX2 in survivors and nonsurvivors with IPAH and controls.

A. Expression of RUNX2 in patients with IPAH and controls. B. Sex differences of RUNX2 in survivors and nonsurvivors with IPAH. Note. IPAH = idiopathic pulmonary arterial hypertension, RUNX2 = runt-related transcription factor 2.

determine its strength in predicting the elevation of mPAP or PVR, as well as reduction of CO. It was found that plasma RUNX2 levels were not only an independent predictor of mPAP elevation, but also an independent index of PVR increase in patients with IPAH ( $p = .029$  and  $p = .001$ ; Table 2).

### Factors Influencing Survival

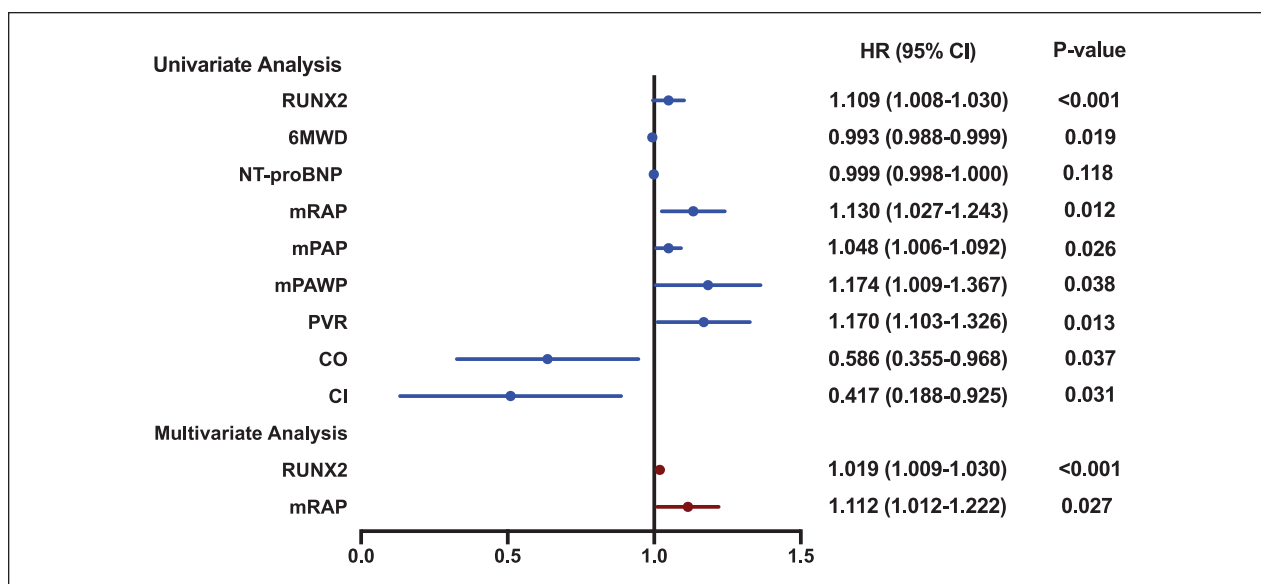
In the univariate cox proportional hazards analysis, RUNX2, 6MWD, mRAP, mPAP, mPAWP, PVR, CO, and CI were significantly related to the survival of all patients with IPAH ( $p < .001$ ,  $p = .019$ ,  $p = .012$ ,  $p = .026$ ,  $p = .038$ ,  $p = .013$ ,  $p = .037$ , and  $p = .031$ ; Figure 2). In the multivariate forward stepwise cox proportional hazards analysis, RUNX2 and mRAP were found to be independent predictors of survival in all patients after adjusting age, gender, and BMI ( $p < .001$  and  $p = .019$ ; Figure 2). Therefore, RUNX2 was a significant player in predicting survival in this cohort.

**Table 2.** RUNX2 as a Determinant of Hemodynamic Parameters in Patients With IPAH.

Dependent parameter	Univariate linear regression				Multivariate linear regression*			
	R	Unstandardized B	95% CI	p value	R	Unstandardized B	95% CI	p value
mRAP	0.005	-0.001	[-0.031,-0.030]	.972				
mPAP	0.324	0.076	[0.009, 0.144]	.028	0.329	0.079	0.008 ~ 0.149	.029
mPAWP	0.109	0.008	[-0.015,0.031]	.471				
PVR	0.491	0.047	[0.022,0.072]	.001	0.492	0.047	0.021 ~ 0.073	.001
CO	-0.297	-0.008	[-0.016, 0.000]	.045				
CI	-0.296	-0.005	[-0.009, 0.000]	.051				

Note. \*Adjusted for age, sex, and BMI.

6MWD = 6-minute walk distance, BMI = body mass index, CI = cardiac index, CO = cardiac output, IPAH = idiopathic pulmonary arterial hypertension, mPAP = mean pulmonary arterial pressure, mPAWP = mean pulmonary capillary wedge pressure, mRAP = mean right atrial pressure, PVR = pulmonary vascular resistance, RUNX2 = runt-related transcription factor 2.



**Figure 2.** Univariate and multivariate cox proportional hazards analysis relating survival to selected RUNX2 levels.

Note. Multivariate analysis adjusted by age, sex, and BMI. 6MWD = 6-minute walk distance, BMI = body mass index, CI = cardiac index, CO = cardiac output, mPAP = mean pulmonary arterial pressure, mPAWP = mean pulmonary capillary wedge pressure, mRAP = mean right atrial pressure, PVR = pulmonary vascular resistance, RUNX2 = runt-related transcription factor 2.

**Table 3.** Receiver-Operating Characteristics.

Variables	Cut-off value	Sensitivity	Specificity	AUC	95% CI	p value
RUNX2	41.5 ng/ml	0.800	0.742	0.819	[0.672, 0.966]	.001
mRAP	3.5 mmHg	0.800	0.677	0.708	[0.535,0.880]	.024

Note. AUC = area under curve, CI = confidence interval, mRAP = mean right atrial pressure, RUNX2 = runt-related transcription factor 2.

**Receiver-Operating Characteristic and Kaplan–Meier Survival Analysis**

ROC analysis for  $34.7 \pm 15.8$  months showed that RUNX2 levels  $\geq 41.5$  ng/ml had a sensitivity of 80.0% and

a specificity of 74.2%, and mRAP  $\geq 3.5$  mmHg had a sensitivity of 80.0% and a specificity of 67.7% in predicting survival in all subjects ( $p = .001$  and  $p = .024$ ; Table 3). Using this as the cut-off value for the Kaplan–Meier analysis, patients with levels of circulating RUNX2  $< 41.5$  ng/ml

had a significantly better prognosis than those with circulating RUNX2  $\geq 41.5$  ng/ml (2-year survival: 92.3% vs. 55.0%; 5-year survival: 88.5% vs. 40.0%, respectively;  $p < .001$ ; Figure 3A).

Patients with mRAP  $< 3.5$  mmHg also had a better survival rate than those with higher mRAP (2-year survival: 95.8% vs. 54.5%; 5-year survival: 87.5% vs. 45.4%, respectively;  $p = .002$ ; Figure 3B).

Regarding influence of gender on survival, male patients with lower RUNX2 levels had a significantly better survival than those with higher RUNX2 levels (2-year survival: 92.3% vs. 60.0%; 5-year survival: 84.6% vs. 20.0%, respectively,  $p = .004$ ; Figure 3C). Similar results were found in female patients (2-year survival: 92.3% vs. 66.7%; 5-year survival: 92.3% vs. 46.7%, respectively,  $p = .015$ ; Figure 3E). No significant difference was observed in survival between male patients with higher and lower mRAP in this cohort (Figure 3D). In contrast, female patients with lower mRAP had a significantly better prognosis than those with higher mRAP (2-year survival: 93.7% vs. 50.0%; 5-year survival: 87.5% vs. 41.7%, respectively,  $p = .009$ ; Figure 3F).

The combination of RUNX2 and mRAP identified subgroups with significantly different survival rates. The group with lower RUNX2 and mRAP had the best survival, whereas the group with higher RUNX2 and mRAP had the worst survival in all patients ( $p < .001$ ; Figure 3D). Similar results were observed in both male and female patients ( $p = .001$  and  $p < .001$ ; Figure 3H and I).

## Discussion

The present study found that levels of circulating RUNX2 in nonsurviving patients with IPAH were higher than in surviving patients with IPAH. There was a positive correlation between levels of plasma RUNX2 and mPAP, mPAWP, as well as PVR, and a negative correlation between RUNX2 and CO. In addition, circulating RUNX2 in combination with mRAP may influence grading of clinical severity and prognosis of patients with IPAH. It was found that patients with a RUNX2 level  $\geq 41.5$  ng/ml and/or mRAP  $\geq 3.5$  mmHg had a significantly poorer prognosis when all patients were taken into consideration as well as when male or female patients were analyzed individually. This suggests that RUNX2 is a powerful predictor of increased cardio-pulmonary vascular risk.

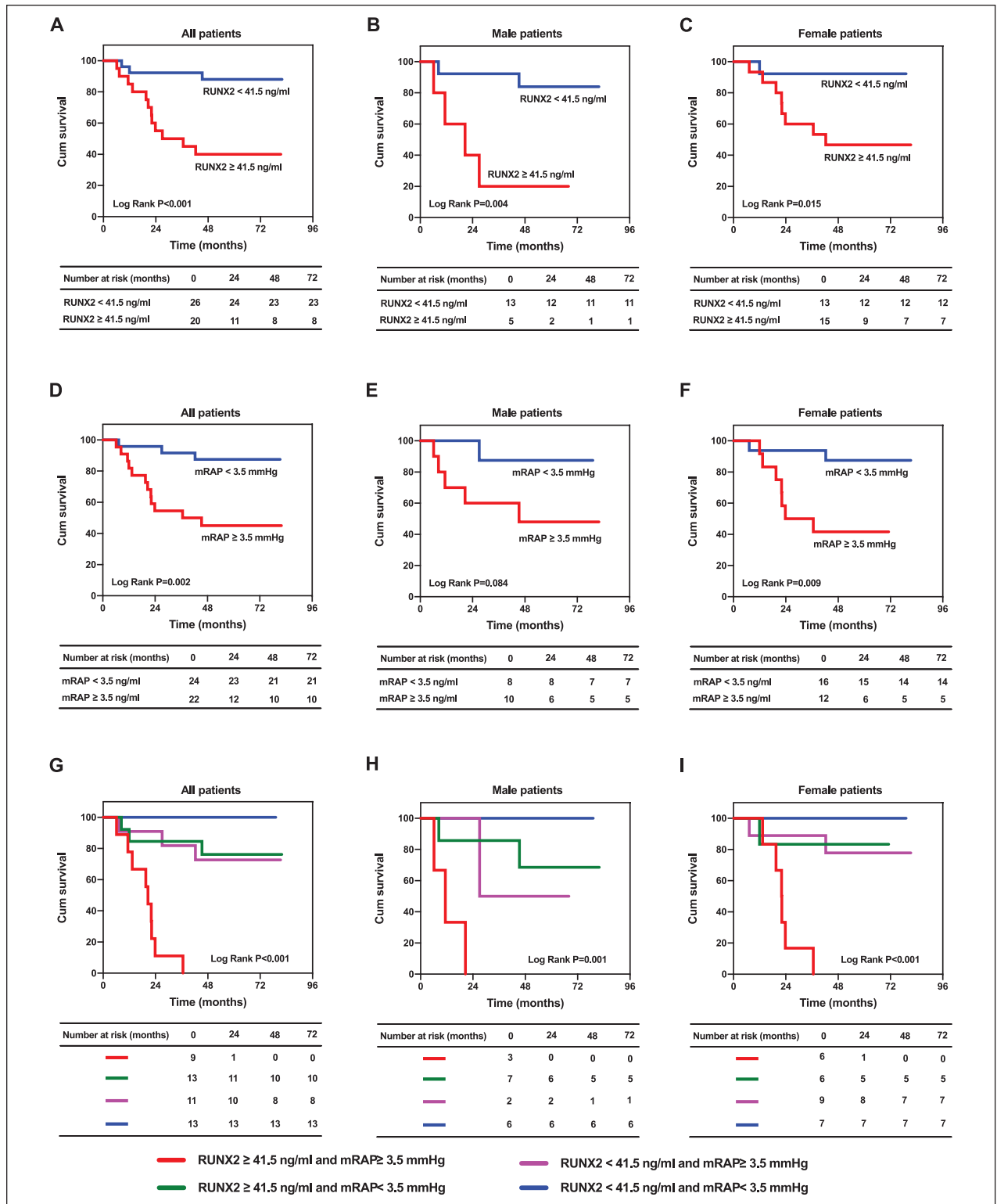
Nonsurviving patients in the present study had subjective and objective features of more severe IPAH, including reduced levels of 6MWD, higher levels of NT-proBNP, and more prominent hemodynamic parameters. Similar findings were reported in cardiovascular calcification (Niederhoffer et al., 1997; Vlachopoulos et al., 2010), suggesting that nonsurvivors might have more pulmonary arterial remodeling and vascular calcification which

contribute to pulmonary arterial complications and mortality independent of traditional cardio-pulmonary vascular risk factors for IPAH. Previous studies have indicated that RUNX2 has an essential role in oxidative stress-induced vascular smooth muscle cell calcification (Byon et al., 2008; Schroeder et al., 2005). Ruffenach et al. (2016) demonstrated that RUNX2 over-expression contributed to pulmonary artery remodeling and stiffness though its expression was very low in normal vascular cells, suggesting that RUNX2 may be an important player in vascular lesion development, including calcification in PAH. Therefore, increased levels of RUNX2 are a marker of pulmonary artery remodeling and calcification.

Hemodynamic parameters are recognized as the most important direct indicators of pulmonary vascular function. It was found that increased plasma RUNX2 levels had significant correlations with mPAP, PVR, and CO in the cohort. However, increased RUNX2 levels can only independently predict the elevation of mPAP and PVR, but not the decline of CO. This means that an increased plasma RUNX2 level has a greater impact on pulmonary artery injury than on the decline of heart function. This result should be interpreted with caution because pulmonary artery injury is manifested earlier than right ventricular dysfunction and right heart failure. Therefore, increased levels of RUNX2 seem to be more responsible for early pulmonary artery lesions in the current cohort.

Prognosis assessment for patients with IPAH is crucial because it determines the type of medical therapies these patients receive and the appropriate timing of referral for lung transplantation. Among noninvasive variables measured in the present study, plasma RUNX2 levels were independently related to survival of patients with IPAH in the cox proportional hazards analysis. This suggests that RUNX2 might provide additional prognostic information than conventional noninvasive assessment. The Kaplan–Meier survival curves using the cut-off value of RUNX2 demonstrated that patients with lower RUNX2 levels had a significantly better survival rate than those with high RUNX2 levels. Thus, plasma RUNX2 might represent a new biomarker for patients with IPAH different from traditionally used markers such as natriuretic peptides and uric acid (Al-Naamani et al., 2016; Nagaya et al., 1999; Torbicki et al., 2003). Future studies with larger sample sizes and biomarker panels are required to clarify their roles in assessing the severity and prognosis of PAH. If confirmed, RUNX2 can be used as a predictor of mortality and a biomarker for treatment response during the long-term follow-up of patients with PAH, since it can be measured routinely and inexpensively.

A marked sex difference in RUNX2 levels was observed in patients with IPAH. Females, including survivors and non-survivors, had higher RUNX2 levels than males. This suggests that females with IPAH might have aggravated vascular calcification. This raises a concern



**Figure 3.** Kaplan–Meier analysis in patients with IPAH based on RUNX2 and mRAP.

A. Survival of patients with IPAH according to the cut-off value of RUNX2 levels. B. Survival of patients with IPAH according to the cut-off value of mRAP. C. Survival of male patients with IPAH according to the cut-off value of RUNX2 levels. D. Survival of male patients with IPAH according to the cut-off value of mRAP. E. Survival of female patients with IPAH according to the cut-off value of RUNX2 levels. F. Survival of female patients with IPAH according to the cut-off value of mRAP. G. Survival of patients with IPAH according to the cut-off value of RUNX2 levels combined with mRAP. H. Survival of male patients with IPAH according to the cut-off value of RUNX2 levels combined with mRAP. I. Survival of female patients with IPAH according to the cut-off value of RUNX2 levels combined with mRAP.

that different sex hormones might potentiate or ameliorate vascular calcification. Our previous studies demonstrated that sex hormones, such as estradiol, testosterone, and progesterone, were associated with an increased risk of PAH (Wu et al., 2018; Zhang et al., 2020). Furthermore, higher levels of estradiol exacerbate the initiation and progression of PAH. Estradiol plays its biological role by binding to the estrogen receptor (ER). A previous study indicated that ER $\alpha$  gene has a RUNX2 binding site within its promoter, and ER $\alpha$  interacts directly with RUNX2 and regulates its activity (Wysokinski et al., 2015). Therefore, females have higher levels of estrogen and ER, which may lead to higher RUNX2 levels in female patients with IPAH. Further studies are needed to elucidate the origin and mechanisms of how sex hormones lead to increased RUNX2 levels in patients with IPAH.

There are a number of limitations in the present study. Firstly, the number of patients included for analysis is relatively small and excluding patients with PAH other than IPAH results in a possible selection bias. Nevertheless, previous studies have described significantly higher RUNX2 levels in patients with PAH. Therefore, similar results are expected if patients with other types of PAH have been included. Secondly, the present study investigated the power of RUNX2 in predicting survival in male and female patients for the first time, but did not analyze the correlation between sex hormones and RUNX2 levels. Thirdly, other factors, including ethnicity and genetic predisposition which could influence metabolic and exercise capacity were not considered in the present study.

## Conclusion

Levels of plasma RUNX2 are positively correlated with the severity of IPAH and high levels of plasma RUNX2 are independently associated with an increased risk for mortality. Future studies with larger sample sizes are needed to confirm our findings.

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## Author Contributions

Ping Yuan and Qian Liu designed the study; Xuntao Yuan, Zuogang Wang, Lan Wang, and Qinhua Zhao collected data. Xuntao Yuan, Zuogang Wang, Sugang Gong performed data analysis and drafted the manuscript. Yuanyuan Sun and Ping Yuan contributed to critical revision of the manuscript; Ping Yuan and Qian Liu contributed to the final revision of the manuscript. All authors approved the final version of the manuscript for submission.

## Declaration of Conflicting Interests

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## References

- Al-Naamani, N., Palevsky, H. I., Lederer, D. J., Horn, E. M., Mathai, S. C., Roberts, K. E., Tracy, R. P., Hassoun, P. M., Girgis, R. E., Shimbo, D., Post, W. S., & Kawut, S. M., & ASA-STAT Study Group. (2016). Prognostic significance of biomarkers in pulmonary arterial hypertension. *Annals of the American Thoracic Society*, 13(1), 25–30. doi: 10.1513/AnnalsATS.201508-543OC.
- American Thoracic Society. (2002). ATS statement: Guidelines for the six-minute walk test. *American Journal of Respiratory and Critical Care Medicine*, 166(1), 111–117. doi: 10.1164/ajrccm.166.1.at1102.
- Benza, R. L., Miller, D. P., Barst, R. J., Badesch, D. B., Frost, A. E., & McGoon, M. D. (2012). An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the reveal registry. *Chest*, 142(2), 448–456. doi: 10.1378/chest.11-1460.
- Byon, C. H., Javed, A., Dai, Q., Kappes, J. C., Clemens, T. L., Darley-Usmar, V. M., McDonald, J. M., & Chen, Y. (2008). Oxidative stress induces vascular calcification through modulation of the osteogenic transcription factor Runx2 by AKT signaling. *Journal of Biological Chemistry*, 283(22), 15319–15327. doi: 10.1074/jbc.M800021200.
- Courboulin, A., Paulin, R., Giguère, N. J., Saksouk, N., Perreault, T., Meloche, J., Paquet, E. R., Biardel, S., Provencher, S., Côté, J., Simard, M. J., & Bonnet, S. (2011). Role for miR-204 in human pulmonary arterial hypertension. *Journal of Experimental Medicine*, 208(3), 535–548. doi: 10.1084/jem.20101812.
- Federici, C., Drake, K. M., Rigelsky, C. M., McNelly, L. N., Meade, S. L., Comhair, S. A., Erzurum, S. C., & Aldred, M. A. (2015). Increased mutagen sensitivity and DNA damage in pulmonary arterial hypertension. *American Journal of Respiratory and Critical Care Medicine*, 192(2), 219–228. doi: 10.1164/rccm.201411-2128OC.
- Galiè, N., Corris, P. A., Frost, A., Girgis, R. E., Granton, J., Jing, Z. C., Klepetko, W., McGoon, M. D., McLaughlin, V. V., Preston, I. R., Rubin, L. J., Sandoval, J., Seeger, W., & Keogh, A. (2013). Updated treatment algorithm of pulmonary arterial hypertension. *Journal of the American College*



- of *Cardiology*, 62(25 Suppl), D60–D72. doi: 10.1016/j.jacc.2013.10.031.
- Galiè, N., Humbert, M., Vachiery, J. L., Gibbs, S., Lang, I., Torbicki, A., Simonneau, G., Peacock, A., Vonk Noordegraaf, A., Beghetti, M., Ghofrani, A., Sanchez, M. A. G., Hansmann, G., Klepetko, W., Lancellotti, P., Matucci, M., McDonagh, T., Pierard, L. A., Trindade, P. T., Zompatori, M., & Hoeper, M., & ESC Scientific Document Group. (2016). 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint 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: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *European Heart Journal*, 37(1), 67–119. doi: 10.1093/eurheartj/ehv317.
- Humbert, M., Sitbon, O., Chaouat, A., Bertocchi, M., Habib, G., Gressin, V., Yaici, A., Weitzenblum, E., Cordier, J.-F., Chabot, F., Dromer, C., Pison, C., Reynaud-Gaubert, M., Haloun, A., Laurent, M., Hachulla, E., & Simonneau, G. (2006). Pulmonary arterial hypertension in France: Results from a national registry. *American Journal of Respiratory and Critical Care Medicine*, 173(12), 1023–1030. doi: 10.1164/rccm.200510-1668OC.
- Jing, Z. C., Jiang, X., Han, Z. Y., Xu, X. Q., Wang, Y., Wu, Y., Lv, H., Ma, C.-R., Yang, Y.-J., & Pu, J. L. (2009). Iloprost for pulmonary vasodilator testing in idiopathic pulmonary arterial hypertension. *European Respiratory Journal*, 33(6), 1354–1360. doi: 10.1183/09031936.00169608.
- Jing, Z. C., Xu, X. Q., Han, Z. Y., Wu, Y., Deng, K. W., Wang, H., Wang, Z.-W., Cheng, X.-S., Xu, B., Hu, S.-S., Hui, R.-T., & Yang, Y. J. (2007). Registry and survival study in Chinese patients with idiopathic and familial pulmonary arterial hypertension. *Chest*, 132(2), 373–379. doi: 10.1378/chest.06-2913.
- Komori, T. (2010). Regulation of bone development and extracellular matrix protein genes by RUNX2. *Cell and Tissue Research*, 339(1), 189–195. doi: 10.1007/s00441-009-0832-8.
- Manes, A., Palazzini, M., Leci, E., Bacchi Reggiani, M. L., Branzi, A., & Galie, N. (2014). Current era survival of patients with pulmonary arterial hypertension associated with congenital heart disease: A comparison between clinical subgroups. *European Heart Journal*, 35(11), 716–724. doi: 10.1093/eurheartj/ehv072.
- Meloche, J., Pflieger, A., Vaillancourt, M., Paulin, R., Potus, F., Zervopoulos, S., Graydon, C., Courboulain, A., Breuils-Bonnet, S., Tremblay, E., Couture, C., Michelakis, E. D., Provencher, S., & Bonnet, S. (2014). Role for DNA damage signaling in pulmonary arterial hypertension. *Circulation*, 129(7), 786–797. doi:10.1161/CIRCULATIONAHA.113.006167.
- Nagaya, N., Uematsu, M., Satoh, T., Kyotani, S., Sakamaki, F., Nakanishi, N., Yamagishi, M., Kunieda, T., & Miyatake, K. (1999). Serum uric acid levels correlate with the severity and the mortality of primary pulmonary hypertension. *American Journal of Respiratory and Critical Care Medicine*, 160(2), 487–492. doi: 10.1164/ajrccm.160.2.9812078.
- Niederhoffer, N., Lartaud-Idjouadiene, I., Giummelly, P., Duvivier, C., Peslin, R., & Atkinson, J. (1997). Calcification of medial elastic fibers and aortic elasticity. *Hypertension*, 29(4), 999–1006. doi: 10.1161/01.hyp.29.4.999.
- Ruffenach, G., Chabot, S., Tanguay, V. F., Courboulain, A., Boucherat, O., Potus, F., Meloche, J., Pflieger, A., Breuils-Bonnet, S., Nadeau, V., Paradis, R., Tremblay, E., Girerd, B., Hautefort, A., Montani, D., Fadel, E., Dorfmueller, P., Humbert, M., Perros, F., . . . Bonnet, S. (2016). Role for runt-related transcription factor 2 in proliferative and calcified vascular lesions in pulmonary arterial hypertension. *American Journal of Respiratory and Critical Care Medicine*, 194(10), 1273–1285. doi: 10.1164/rccm.201512-2380OC.
- Schroeder, T. M., Jensen, E. D., & Westendorf, J. J. (2005). Runx2: A master organizer of gene transcription in developing and maturing osteoblasts. *Birth Defects Research Part C: Embryo Today*, 75(3), 213–225. doi: 10.1002/bdrc.20043.
- Sutendra, G., Dromparis, P., Wright, P., Bonnet, S., Haromy, A., Hao, Z., McMurtry, M. S., Michalak, M., Vance, J. E., Sessa, W. C., & Michelakis, E. D. (2011). The role of Nogo and the mitochondria-endoplasmic reticulum unit in pulmonary hypertension. *Science Translational Medicine*, 3(88), 88ra55. doi: 10.1126/scitranslmed.3002194.
- Torbicki, A., Kurzyna, M., Kuca, P., Fijałkowska, A., Sikora, J., Florczyk, M., Pruszczyk, P., Burakowski, J., & Wawrzyńska, L. (2003). Detectable serum cardiac troponin T as a marker of poor prognosis among patients with chronic precapillary pulmonary hypertension. *Circulation*, 108(7), 844–888. doi: 10.1161/01.CIR.0000084544.54513.E2.
- Vlachopoulos, C., Aznaouridis, K., & Stefanadis, C. (2010). Prediction of cardiovascular events and all-cause mortality with arterial stiffness: A systematic review and meta-analysis. *Journal of the American College of Cardiology*, 55(13), 1318–1327. doi:10.1016/j.jacc.2009.10.061.
- Wu, W. H., Yuan, P., Zhang, S. J., Jiang, X., Wu, C., Li, Y., Liu, S.-F., Liu, Q.-Q., Li, J.-H., Pudasaini, B., Hu, Q.-H., Dupuis, J., & Jing, Z. C. (2018). Impact of pituitary-gonadal axis hormones on pulmonary arterial hypertension in men. *Hypertension*, 72(1), 151–158. doi: 10.1161/HYPERTENSIONAHA.118.10963.
- Wysokinski, D., Blasiak, J., & Pawlowska, E. (2015). Role of RUNX2 in breast carcinogenesis. *International Journal of Molecular Sciences*, 16(9), 20969–20993. doi: 10.3390/ijms160920969.
- Zhang, Y. X., Wang, L., Lu, W. Z., Yuan, P., Wu, W. H., Zhou, Y. P., Zhao, Q.-H., Zhang, S.-J., Li, Y., Wu, T., Jiang, X., & Jing, Z. C. (2020). Association between high FSH, low progesterone, and idiopathic pulmonary arterial hypertension in women of reproductive age. *American Journal of Hypertension*, 33(1), 99–105. doi: 10.1093/ajh/hpz143.