

GDF15 is a Putative Biomarker for Distinguishing Pulmonary Veno-Occlusive Disease and Pulmonary Arterial Hypertension

Amit Prabhakar¹, Eckart MDD De Bie^{2,3}, Jacqueline DesJardin⁴, Prajakta Ghatpande¹, Stefan Gräf², Luke S. Howard^{5,6}, S. John Wort^{5,7}, Colin Church⁸, David G. Kiely⁹, UK Pulmonary Arterial Hypertension Cohort Study Consortium, Emily Sumpena⁴, Thin Aung⁴, Shenrae Carter¹⁰, Jasleen Kukreja¹⁰, Steven Hays¹⁰, John R. Greenland^{10,11}, Jonathan P. Singer¹⁰, Michael Wax¹⁰, Paul J. Wolters¹⁰, Marc A. Simon⁴, Mark Toshner^{2,3}, Giorgio Lagna¹, Akiko Hata^{1,12}

1. Cardiovascular Research Institute, University of California, San Francisco (UCSF), San Francisco, CA, USA
2. Victor Phillip Dahdaleh Heart & Lung Research Institute, University of Cambridge, Cambridge, UK
3. Royal Papworth Hospital NHS Foundation Trust, Cambridge, UK
4. Pulmonary Vascular Disease, PAH Center of Comprehensive Care, Division of Cardiology, Department of Medicine, UCSF, San Francisco, CA, USA
5. Imperial College London Faculty of Medicine, National Heart & Lung Institute, London, UK and Northern Ireland
6. Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, UK
7. Royal Brompton Hospital, Adult Centre for Pulmonary Hypertension, London, UK and Northern Ireland
8. Golden Jubilee National University Hospital and University of Glasgow, Glasgow, Scotland
9. Sheffield Pulmonary Vascular Disease Unit and NIHR Biomedical Research Centre, Sheffield Teaching Hospitals NHS Foundation Trust, UK and Clinical Medicine, University of Sheffield, Sheffield, UK
10. Pulmonary, Critical Care, Allergy and Sleep Medicine, UCSF, San Francisco, CA, USA
11. San Francisco VA Health Care System, San Francisco, CA, USA
12. Department of Biochemistry and Biophysics, UCSF, San Francisco, CA, USA

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To the Editor: Pulmonary veno-occlusive disease (PVOD) is a rare, severe Group 1 pulmonary arterial hypertension (PAH) subtype with poor survival (1). PAH-targeted vasodilators can cause life-threatening pulmonary edema in PVOD, underscoring the need for diagnostic tools to distinguish it from other PAH subtypes (1). In patients and mitomycin C (MMC)-induced model animals, aberrant activation of integrated stress response (ISR) via Protein Kinase R (PKR) drives cardiovascular phenotypes of PVOD (2-4). Inhibition of the PKR-ISR axis, using either the PKR inhibitor C16 or the ISR inhibitor ISRIB, reverses PVOD phenotypes (2-4). Because GDF15 is a cytokine induced by the ISR (5), we compared plasma GDF15 levels in rat models of PVOD and PAH to evaluate its potential as a biomarker. Upon MMC treatment, GDF15 mRNA and plasma protein levels increased (**Supplementary Figure 1A**), which was reversed with ISRIB treatment (6) (**Supplementary Figure 1B**). Plasma GDF15 levels were 2.2-fold higher in PVOD model rats compared to those in monocrotaline (MCT)-induced PAH model rats (**Supplementary Figure 1C**), suggesting a potential distinction in circulating GDF15 levels between PVOD and other PAH subtypes.

Plasma GDF15 concentrations in patients with PVOD, idiopathic PAH (IPAH), heritable PAH (HPAH), Group 2-4 PH (Other PH), chronic obstructive pulmonary disease without PH (COPD), and healthy controls (Ctrl) were quantified (**Figure 1A**). The median GDF15 level in PVOD was 12.4-, 3.0-, 2.4-, 2.1-, and 2.4-fold higher than in Ctrl, IPAH, HPAH, Other PH, and COPD, respectively (**Figure 1A**). No significant sex-dependent differences in GDF15 were observed (**Supplementary Figure 2A**). ROC analysis showed that PVOD had the highest AUC among all cohorts with both sensitivity

and specificity of 100% (**Figure 1B**), whereas specificity for IPAH, HPAH, Other PH, and COPD was lower at the same sensitivity (**Figure 1C**). It also showed that plasma GDF15 levels distinguished PVOD from other cohorts with AUC values of 94% or higher (**Figure 1D**). At 100% specificity for PVOD, GDF15 maintained a specificity greater than 58% when compared to other cohorts (**Figure 1D-F**). GDF15 also distinguished PVOD from the combined IPAH+HPAH+Other PH and IPAH+HPAH cohorts with an optimal cutoff value of 1,658 pg/ml with 81% sensitivity and 98% specificity. GDF15 levels were significantly associated with PVOD compared to all reference cohorts (**Figure 1E**). An inverse correlation was observed between age-adjusted GDF15 and 6-minute walk distance (6MWD) in the PVOD+IPAH+HPAH cohort, indicating an association with disease severity (**Figure 1G**); however, no such correlation was observed in the PVOD cohort alone (**Supplementary Figure 2B**). Age-adjusted GDF15 (**Figure 1H**), but not other parameters (**Figure 1I-M**), differentiated PVOD from IPAH and HPAH. Higher levels of GDF15 were associated with poorer survival in the PVOD+IPAH+HPAH cohort (**Figure 1N**), with a similar trend observed in the PVOD cohort (**Supplementary Figure 2D**). Our findings suggest that GDF15 may serve as both a diagnostic biomarker to distinguish PVOD from other PAH subtypes and a prognostic biomarker for patients with PVOD, IPAH, and HPAH.

See Supplemental Data for study population characteristics, methods, additional data, author contributions, and acknowledgments.

Address correspondence to: Akiko Hata, 555 Mission Bay Blvd. South, SCVRB Rm 252T, San Francisco, CA 94143, USA. Phone: 415-476-9758;

Email: akiko.hata@ucsf.edu

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Figure legend

Figure 1 Plasma GDF15 as a diagnostic and prognostic biomarker for PVOD.

A. Violin plot of plasma GDF15 concentrations across different cohorts; $P < 0.0001$ by one-way ANOVA with Turkey's post hoc test. **B.** ROC curves comparing GDF15 levels between healthy controls (Ctrl) and individual cohorts. **C.** Specificity% (95% CI) at 100% sensitivity for distinguishing Ctrl from individual cohorts. **D.** ROC curves for distinguishing PVOD from individual cohorts. **E.** ROC curves comparing PVOD with combined cohorts: IPAH+HPAH+Other PH or IPAH+HPAH. **F.** Specificity% (95% CI) at 100% sensitivity for distinguishing PVOD from individual or combined cohorts. **G.** Pearson correlation between age-adjusted GDF15 and 6MWD in the combined cohort: PVOD+IPAH+HPAH. **H-M.** Standardized distributions of diagnostic parameters: age-adjusted GDF15 (**H**), NT-proBNP (**I**), pulmonary vascular resistance (PVR) (**J**), mean

pulmonary arterial pressure (mPAP) (**K**), cardiac index (CI) (**L**), and 6MWD (**M**) in PVOD, IPAHA, and HPAHA. Left: individual values normalized to the median ($y=0$). Right: median values with 95% CI. **N**. Cox proportional hazards model showing the association between plasma GDF15 and transplant-free survival in the combined cohort: PVOD+IPAHA+HPAHA, adjusted for age and sex.

