The angiogenic gene profile of pulmonary endarterectomy specimens: Initial study

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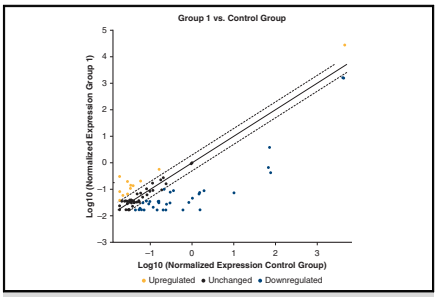
ABSTRACT

Objectives: The underlying mechanisms for the development of chronic thromboembolic pulmonary hypertension and prognostic biomarkers are not clear yet. Thus, our aim is to assess and identify new biomarkers for the expression of 84 key genes linked to angiogenesis.

Methods: Patients who had levels more than 1000 dynes·sec·cm^{-5} were included in the test group, and the other patients were included in the control group. Twelve specimens were taken from the patients. RT^2 Profiler PCR Array (Qiagen) was used to quantify the expression of the 84 key genes.

Results: Eight patients (6 male, 2 female, median age 54.4 ± 13.1 years) who underwent pulmonary endarterectomy were included. Pulmonary vascular resistance improved significantly from 811 ± 390 dyn/s/cm^{-5} to 413.3 ± 144.9 dyn/s/cm^{-5} ($P < .005$). A difference was also detected in median mean pulmonary arterial pressure, which decreased from 49.8 ± 9 mm Hg to 32.62 ± 2.50 mm Hg ($P > .005$) after surgery. Median length of hospital stay was 11.62 ± 2.97 days. The test group had a distinct pattern of impaired angiogenic and antiangiogenic genes. The expression levels of TGFA, TGFB1, THBS2, THBS1, TGFBR1, SERPINE1, SERPINF1, TGFB2, TIMP2, VEGFC, IFNA1, TNF, CXCL10, NOS3, IGF1, and MMP14 were downregulated in the specimens from the patients who had higher pulmonary vascular resistance values, whereas some genes, including PDGFA, showed upregulation that was statistically nonsignificant in the same group.

Conclusions: These results can lead to the development of new markers that could predict adverse outcomes of patients with CTEPH. Identification of new markers that are related to worse outcomes would enable screening patients for early diagnosis and treatment. (JTCVS Open 2023;■:1-12)



A total of 38 of 84 genes were altered in the test group when compared with the control group.

CENTRAL MESSAGE

Angiogenic and antiangiogenic factors in patients with CTEPH play an important role. Identification of the factors that are correlated with worse outcomes might be useful for screening preoperatively.

PERSPECTIVE

The underlying mechanism leading to CTEPH is still controversial. Angiogenic and antiangiogenic factors have a major impact on the pathophysiology of the disease. It is an important step to identify those factors, especially the ones paving the way for severe disease. Thus, it will become possible to screen and detect those patients to establish a precautious treatment plan.

See Commentary on page XXX.

Video clip is available online.

Chronic thromboembolic pulmonary hypertension (CTEPH) is a late complication of acute venous thromboembolism obstructing the pulmonary arteries. Although

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Abbreviations and Acronyms

CT = cycle threshold
CTEPH = chronic thromboembolic pulmonary hypertension
ECM = extracellular membrane
MMP = matrix metalloproteinase
mRNA = messenger RNA
PA-1 = plasminogen activator inhibitor-1
PEA = pulmonary endarterectomy
PEDF = pigment epithelium-derived factor
PH = pulmonary hypertension
PVR = pulmonary vascular resistance
TGF = transforming growth factor
VEGF = vascular endothelial growth factor

these patients receive anticoagulation therapy for at least 3 months, mean pulmonary arterial pressure has been documented as more than 25 mm Hg.[1,2] These outcomes lead to severe right ventricular dysfunction, which might be fatal. Pulmonary endarterectomy (PEA) is the recommended gold treatment for patients with CTEPH.[3]

There is a period of several months or years between acute pulmonary embolism and beginning of the symptoms of CTEPH.[4] Pathogenesis of the development of CTEPH after acute pulmonary embolism is still poorly understood, and it is believed there is a complex interaction between thrombotic/thrombolytic processes and angiogenic response during this transformation.[5,6] Even if some angiogenic and antiangiogenic biomarkers have been shown to be altered in patients with CTEPH in previous studies, the underlying mechanism and prognostic biomarkers of this disease are controversial.[7-9] We identified our null hypothesis as no relationship exists between angiogenetic/antiangiogenetic gene expressions and CTEPH in terms of disease severity and worse outcomes. Thus, we screened PEA samples from the patients who had higher and lower PVR levels for the expression of 84 key genes linked to angiogenesis (Figure 1).

MATERIALS AND METHODS

Eight consecutive patients with the diagnosis of CTEPH who underwent PEA at our center were included. CTEPH was diagnosed by the presence of mismatched perfusion defects on the radioisotopic ventilation perfusion scan associated with evidence of pulmonary hypertension (PH) on transthoracic echocardiogram despite adequate anticoagulation for at least 3 months. Pulmonary function tests, computed tomography pulmonary angiography, 6-minute walk test, and right heart catheterization were performed as routine preoperative workups for all patients. Cardiac output was determined by thermodilution, and pulmonary vascular resistance (PVR) was calculated. The patients were grouped according to their PVR levels. Among 8 patients, 5 had PVR levels less than 1000 dynes·sec·cm^{-5} and were included in the control group. It was well documented by Dartevelle and colleagues[9] that patients with increased PVR have higher mortality rates than patients with PVR less than 900 dynes/s/cm^{-5}. A higher postoperative mortality rate occurs if PEA cannot reduce the pulmonary resistance by 50%. Three of the patients who had PVR levels more than 1000 dynes·sec·cm^{-5} were included in the test group. Figure 2 outlines the patient flow. The demographics and preoperative characteristics of the patients are shown in Table 1. The following tests are routinely performed during assessment for surgery: erythrocyte sedimentation rate, C-reactive protein, complete blood count, and blood chemistry including renal, liver, thyroid function, and urinalysis tests. Antinuclear antibodies, extractable nuclear antigen panel, antineutrophil cytoplasmic antibodies, antiglomerular basement membrane antibodies, anticardiolipin antibodies, tests for hepatitis B and C, and HIV and complement levels are performed as required. Positron emission tomography computed tomography has been used in patients with suspected pulmonary artery sarcoma or systemic vasculitis. Two patients had systemic lupus erythematosus, 1 patient had Behcet's disease, and 1 patient had hypertension as associated medical problems.

All PEAs were performed under deep hypothermia and intermittent circulatory arrest (20 °C). After cardiopulmonary bypass initiation, it is aimed to cool down the patient gradually to 20 °C core temperature. Once a core temperature of 20 °C is reached, the dissection begins on the right side. After performing an incision in the right pulmonary artery, dissection starts after finding a correct thromboendarterectomy plane. Although the initial dissection can be performed with full cardiopulmonary bypass, once visualization becomes compromised by return of blood flow, dissection cannot proceed safely. At this stage, the circulation needs to be arrested to provide a completely clear field. A crossclamp is placed on the ascending aorta, and total circulatory arrest is initiated on the right side by stopping circulation. After completing pulmonary thromboendarterectomy, the crossclamp is removed and the heart is reperfused. The same steps are followed on the left side. The rewarming phase begins after completion of PEA on both sides (Video 1). Patients are kept intubated and transferred to the intensive care unit, where postoperative hemodynamic parameters and mean pulmonary arterial pressure are closely monitored from the first postoperative day to the transfer from the intensive care unit to the floor. Figure 3 shows preoperative computed tomography pulmonary angiograms and postoperative specimens.

The study was designed and performed with Betül Yilmaz and Saime Batirel, who are professors in Medical Biochemistry and managers and deputy managers of the Genetic and Metabolic Diseases Research and Investigation Center. A total of 12 PEA specimens from the patients were taken during PEA and stored in RNAlater RNA stabilization reagent solution (Qiagen) until measurements of messenger RNA (mRNA) expressions were documented by RT^2 Profiler PCR Array (Qiagen). The study protocol was approved by the Marmara University Ethics and Research Committee (Protocol No. 09.2014.0272 with an approval date of December 18, 2014). A written informed consent was provided from each patient to participate in the study.

RNA Preparation and Complementary DNA Synthesis

The expressions of angiogenesis-related genes were quantified in all specimens to investigate possible differences between the 2 groups. Total RNA was isolated from PEA specimens using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. RNA concentrations were measured with BioSpec-nano spectrophotometer (Shimadzu). Complementary DNA synthesis was performed by using the RT^{2}HT First Strand Kit (Qiagen) according to the manufacturer's protocol.

RT^2 Profiler PCR Array

The Human Angiogenesis RT^2 Profiler PCR Array (Qiagen) was used to quantify the expression of 84 key genes known to be involved in angiogenesis. Complementary DNA was mixed with RT^2 SYBR Green ROX FAST Master Mix (Qiagen) and RNase-free water. This mixture was added to each well, which contained primers. Amplification was performed in the Rotor-Gene-Q real-time PCR cycler (Qiagen) with the cycling conditions

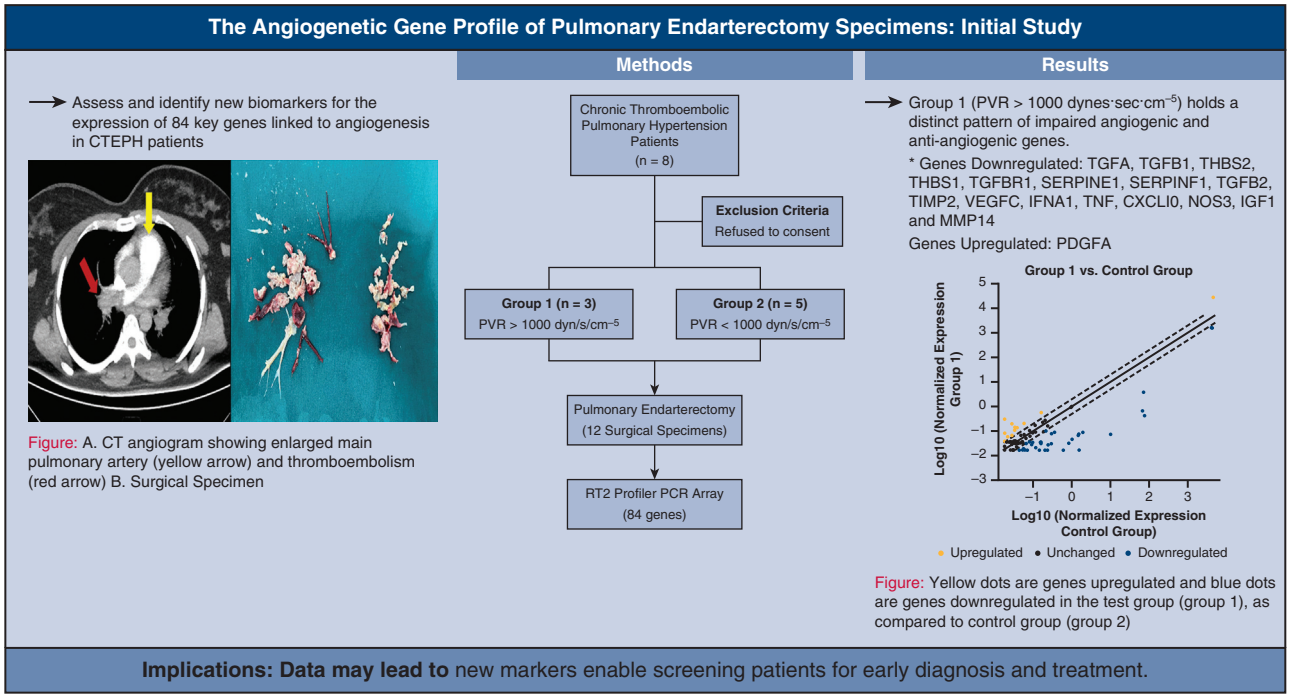


FIGURE 1. Graphical abstract presents a brief explanation of the study. *CTEPH*, Chronic thromboembolic pulmonary hypertension; *PVR*, pulmonary vascular resistance.

of 10 minutes at 95 °C, 15 seconds at 95 °C, and 1 minute at 60 °C for 40 cycles with a final infinite 4 °C hold.

Data Analysis and Statistical Analysis

Raw data from RT^2 Profiler PCR Array were transformed to the cycle threshold (CT) values with Rotor-Gene Series software, version 2.0.2.4 (Qiagen). By using a web-based RT^2 Profiler PCR Array Data Analysis tool (https://www.qiagen.com/tr/shop/genes-and-pathways/data-analysis-center-overview-page/), these CT values were normalized to GAPDH, one of the housekeeping genes, and then the relative gene expression levels were calculated. The control samples were set to 1- and 2-fold or greater change in expression considered abnormal. The 2 groups were compared by using the Student *t* test. The results are shown as mean ± standard deviation.

RESULTS

Six of the patients were male, and 2 patients were female. PVR improved significantly from 811 ± 390 dyn/s/cm^{-5} to 413.3 ± 144.9 dyn/s/cm^{-5} ($P < .005$). A difference was also detected in median mean pulmonary arterial pressure as a decrease from 49.8 ± 9 mm Hg to 32.62 ± 2.50 mm Hg ($P > .005$) after surgery. The median length of hospital stay was 11.62 ± 2.97 days. The patients were divided into 2 groups according to their PVR levels. The median PVR level of 5 subjects in the control group was 745 ± 206 dyn/s/cm^{-5}, and the mean PVR level in the test group was 1500 ± 336 dynes·sec·cm^{-5}, which was significantly higher than in the control group ($P < .05$). Moreover, PVR levels significantly improved after PEA for the study and control groups, decreasing to 503 ± 151 dyn/s/cm^{-5} and 437 ± 176 dyn/s/cm^{-5}, respectively ($P < .05$).

All patients described shortness of breath and fatigue as their chief symptoms. The functional capacity of the patients was New York Heart Association class III or IV, except 1 patient who had class II. Postoperative hemodynamic measurements were monitored and recorded for all patients. Thirty-day mortality was observed in 1 patient (12.5%) who had aspiration pneumonia. Patients received a median follow-up of 8 months[2-14] after the PEA. To date, all the survivors are alive without any recurrence. The functional capacity of the patients improved to New York Heart Association class I after PEA. Moreover, none of them have experienced worsening in symptoms. The intraoperative and postoperative data are summarized in Table 2.

RT^2 Profiler PCR Array Analysis of Angiogenesis

mRNA expressions were tested by the RT^2 Profiler PCR Array including 84 genes in its panel, which is specific for angiogenesis profile. The design of the study was performed according to this array analyzing the data by specific software that guarantees accuracy and reliability.

Twelve PEA specimens from 8 patients who underwent a PEA operation were screened for the expression of 84 genes linked to angiogenesis using RT^2 Profiler PCR Array

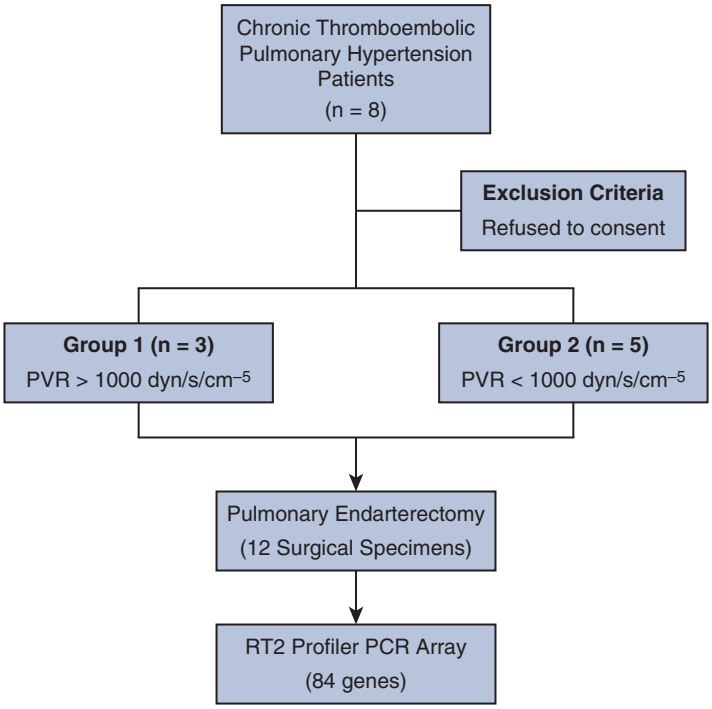


FIGURE 2. Diagram demonstrates the flow of the study. Patients with the diagnosis of CTEPH who underwent PEA at our center were included. The patients were grouped according to their PVR levels. Among 8 patients, 5 had PVR levels less than 1000 dynes·sec·cm^{-5} and were included in the control group. Three of the patients who had PVR levels more than 1000 dynes·sec·cm^{-5} were included in the test group. A total of 12 PEA specimens from 8 patients were taken during PEA for measurements of mRNA expressions by RT^2 Profiler PCR Array. *PVR*, Pulmonary vascular resistance.

analysis. The gene expression levels in PEA specimens from patients whose PVR levels were higher than 1000 dynes·sec·cm^{-5} were compared with the gene expressions of the specimens from patients whose PVR levels were lower than 1000 dyn/s/cm^{-5}. A qualitative cluster analysis of the data was conducted via cluster grams (Figure E1). Data analysis revealed that 38 of 84 genes were altered in the test group compared with the control group. As represented in the scatter plot (Figure 4), 10 of 84 genes showed 2-fold or greater higher expression levels, and 28 of 84 genes showed 2-fold or greater downregulation in the test group. Table 3 shows the list of 84 genes and reports of normalized expression levels as fold regulation. However, fold regulation changes were not found to be statistically significant in the test group compared with the control group. Although there were multiple fold regulation changes between the 2 groups, we did not find a significant difference because of the high standard deviations in the mRNA expression data.

The data suggested that the test group had a distinct pattern of impaired angiogenic and antiangiogenic genes. Most notably, the expression levels of PDGFA, KDR, and CDH5 were upregulated in the specimens from the patients whose PVR levels were higher than 1000 dynes·sec·cm^{-5}. Moreover, 17 genes, including TGFA, TGFB1, THBS2, THBS1, TGFBR1, SERPINE1, SERPINF1, TGFB2, TIMP2, VEGFC, TIMP1, IFNA1, TNF, CXCL10, NOS3, GF1, and MMP14, showed 4-fold or greater downregulation in the same group. Although these 17 genes showed 4-fold or greater downregulation in the test group, there was no significant difference compared with the control group. These findings demonstrated that the gene expressions related to angiogenesis were changed in patients with CTEPH who had a poorer hemodynamic profile.

DISCUSSION

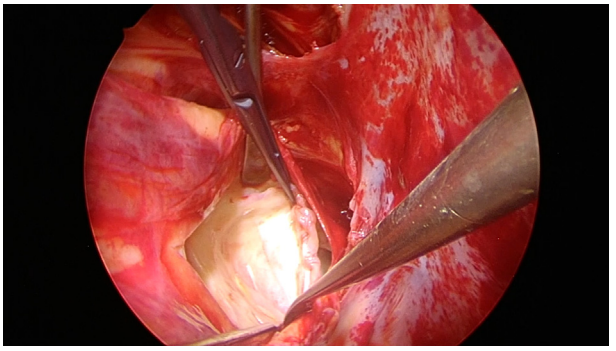
CTEPH is a late complication of PH resulting from an acute venous thromboembolism obstructing the pulmonary arteries.[10-12] PEA is a potentially curative operation for these patients.[3] The pathophysiology in the failure of this resolution of the thromboemboli and development of CTEPH is still not clear. Genetic defects, defective fibrinolysis, prothrombotic factors, and abnormalities in angiogenesis are suggested as the main reasons responsible for the disease.[13] Because of the paucity of vessels and low ratio between thrombus angiogenesis and fibrosis in PEA specimens, it was suggested that an intact angiogenic response is more crucial for thrombus resolution than the fibrinolytic system.[8] Although few studies have investigated the angiogenic and inflammatory background of CTEPH, we still

TABLE 1. Patient demographics and preoperative characteristics

Characteristics	Value or n
Age (y)	54.4 ± 13.1
Sex (n)	
Female	2 (25%)
Male	6 (75%)
Duration from symptom to surgery (mo)	30.1 ± 22.3
Symptoms (n)	
Shortness of breath	8 (100%)
Fatigue	8 (100%)
Cough	5 (62.5%)
Headache	3 (37.5%)
Hemoptysis	1 (12.5%)
NYHA class (n)	
I	0
II	1 (12.5%)
III	5 (62.5%)
IV	2 (25%)
6MWT (m)	226.8 ± 208.8
FEV1 (L)	2.49 ± 1.07
FEV1 (%)	80.75 ± 25.1
mPAP (mm Hg)	49.8 ± 9
Cardiac index (L/min/m^2)	2.62 ± 1.75
Cardiac output (L/min/m^2)	4.42 ± 2.42
PVR (dyn/s/cm^{-5})	811 ± 390
Comorbidities (n)	
SLE	2 (25%)
Behcet's disease	1 (12.5%)
Stroke	1 (12.5%)
Hypertension	1 (12.5%)

Values are presented as a number (the percentage of variables) or the mean and standard deviation. *NYHA*, New York Heart Association; *6MWT*, 6-minute walk test; *FEV_1*, forced expiratory volume in 1 second; *mPAP*, mean pulmonary arterial pressure; *PVR*, pulmonary vascular resistance; *SLE*, systemic lupus erythematosus.

need more data[14,15] (Table E1). This is the first study to determine 84 gene expressions related to angiogenesis in PEA specimens. In this study, we observed that some angiogenic genes were downregulated and some were upregulated in the PEA specimens of patients with CTEPH with higher PVR levels. Vascular endothelial growth factor (VEGF) promotes angiogenesis. The VEGF family consists of isoforms, namely, VEGFA, VEGFB, VEGFC, and VEGFD. It was also observed that plasma concentrations of VEGFA and VEGFD increased in patients with CTEPH compared with controls.[7] On the other hand, VEGFA levels were decreased in these patients after PEA.[16] In addition, injection of VEGF enhanced recanalization and organization on venous thrombi.[17] FLT-1 (VEGFR-1) is the receptor of VEGFA and VEGFB, and KDR (VEGFR-2) is another receptor that is bound by VEGFA, VEGFC, and VEGFD. Both receptors are expressed by endothelial cells,



VIDEO 1. The technical steps of PEA for CTEPH. Video available at: https://www.jtcvs.org/article/S2666-2736(23)00003-7/fulltext.

contribute to the angiogenic response, and play a role in thrombus resolution.[8] Similar to VEGFA, increased plasma concentrations of soluble FLT-1 were found in patients with CTEPH and correlated with worse hemodynamics in patients with PH.[7] In another study, the lack of KDR led to larger thrombus.[8] In our study, we observed higher VEGFA and KDR but lower VEGFB and VEGFC gene expression levels in PEA specimens from patients with higher PVR levels. Endoglin expression was upregulated, which might be caused by endoglin interacting with VEGFR-2 and preventing degradation. Because PDGF implicates endothelial cell dysfunction and proliferation and migration of vascular smooth muscle cells, it was assumed that altered PDGF signaling is involved in the vascular remodeling occurring in pulmonary arterial hypertension.[18] Ogawa and colleagues[19] observed a high deposition of PDGF and its receptor in PEA tissues. Additionally, we found that the patients with higher PVR levels have higher PDGF gene expression levels.

Angiopoietins are other angiogenic molecules. All angiopoietins are the ligands of TIE-2 receptor. Angiopoietin-1 plays a critical role in vessel remodeling and maturation. However, angiopoietin-2 promotes cell death and vascular regression.[20] Upregulated angiopoietin-1 has been found in the lungs of patients with CTEPH.[21] In another study, angiopoietin-2 gene expression was lower in CTEPH thrombi compared with pulmonary arteries.[8] It was also observed that high preoperative levels of angiopoietin-2 were correlated with worse outcomes for PEA.[22] Upregulated angiopoietin-2 expression might indicate impaired angiogenesis in our study. Angiogenesis is a highly controlled process with a good balance between local angiogenic and antiangiogenic factors. Zabini and colleagues[23] wrote an article regarding the role of antiangiogenic factors in patients with CTEPH. THBS-1, one of the antiangiogenic factors, presents within blood vessels and takes a role in maintaining vascular structure and homeostasis. There is a positive feedback loop between THBS-1 and transforming growth factors (TGFs).

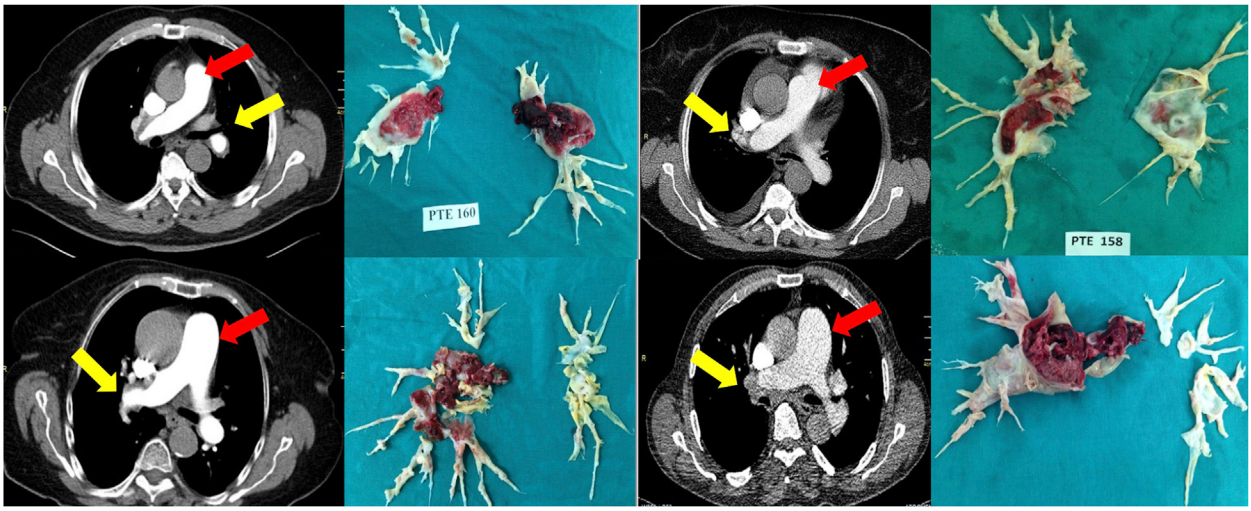


FIGURE 3. Preoperative computed tomography pulmonary angiograms showing enlarged main pulmonary arteries (*red arrows*), significant chronic thromboembolic lesions (*yellow arrows*), and postoperative PEA specimens.

THBS-1 can bind and activate TGF-β1, whereas the expression of THBS-1 is induced by TGF-β1 and TGF-β2. Animal studies showed that TSP-1 is selectively upregulated after ischemia induced by TGF-β and basic fibroblast growth factor-β.[24] Moreover, TGF-β1 expression was found at lower levels in CTEPH thrombi compared with pulmonary arteries.[8] We also found that the gene expressions of TGF-α, TGF-β1, TGF-β2, and TGF-βR1 were downregulated in patients with CTEPH who have poorer hemodynamic measurements. Serpins are involved in many physiological processes, such as blood coagulation, fibrinolysis, and inflammation. We observed that SERPINE1 (plasminogen activator inhibitor-1 [PA-1]) and SERPINF1 (pigment epithelium-derived factor [PEDF]) were downregulated in the test group, and that expression of plasminogen activator was increased in the same group, which is consistent. PA-1 is an inhibitor of fibrinolytic system and affects angiogenesis in a concentration-dependent manner. The lower concentrations of PA-1 were found to be related with angiogenesis. PEDF has antiangiogenic and antithrombogenic effects.[24] Both expressions of serpins were downregulated in the test group of our study. On the other hand, because hypoxia leads to decreased levels of PEDF,[25] hypoxia caused by CTEPH might contribute to this downregulation. Additionally, we found that the alpha subunit of hypoxia-inducible factor-1, which plays a role in ischemic disease, was upregulated.

TABLE 2. Intraoperative and postoperative characteristics

Characteristics	Value or n
CBP (min)	334.25 ± 44.9
Aortic crossclamp (min)	45.25 ± 23.7
TCA (min)	42.75 ± 16.5
MV time (d)	1.87 ± 1.45
ICU (d)	4.5 ± 2.20
LOS (d)	11.62 ± 2.97
Postoperative mPAP (mm Hg)	32.62 ± 2.50
Postoperative PVR (dyn/s/cm^{-5})	413.3 ± 144.9
Postoperative NYHA class I (n)	7 (87.5%)

Values are presented as a number (the percentage of variables) or the mean and standard deviation. *CBP*, Cardiopulmonary bypass; *TCA*, total circulatory arrest; *MV*, mechanical ventilation; *ICU*, intensive care unit; *LOS*, length of stay; *mPAP*, mean pulmonary arterial pressure; *PVR*, pulmonary vascular resistance; *NYHA*, New York Heart Association.

Processes of inflammation and angiogenesis are interconnected. Under the hypoxia state, inflammatory cells secrete angiogenic factors. Additionally, they release matrix metalloproteinase (MMP)s, plasminogen, and urokinase molecules, which contribute to remodeling of the extracellular membrane (ECM) and allow blood vessel formation. Chemokines, such as CXCL12, CXCL8, and CXCL1, are involved in immunoregulatory and inflammatory processes and enhance angiogenesis indirectly.[26] Inflammation is known to play role in the development of CTEPH, and some inflammatory markers such as C-reactive protein, tumor necrosis factor (TNF)-alpha, interleukin-1b, interleukin-2, interleukin-4, interleukin-8, interleukin-10, MMP9, macrophage inflammatory protein-1a, and monocyte chemotactic protein-1 were found increased in plasma and thrombus samples from patients with CTEPH.[6,13] Excessive inflammation disrupts thrombus resolution, which might lead to more frequent CTEPH occurrence; noninfectious inflammatory states also promote stabilization of thromboemboli and increase the risk for the development of CTEPH.[10] Our study showed that some inflammatory markers such as TNF, interferon alpha-1, and interleukin-1 beta were downregulated

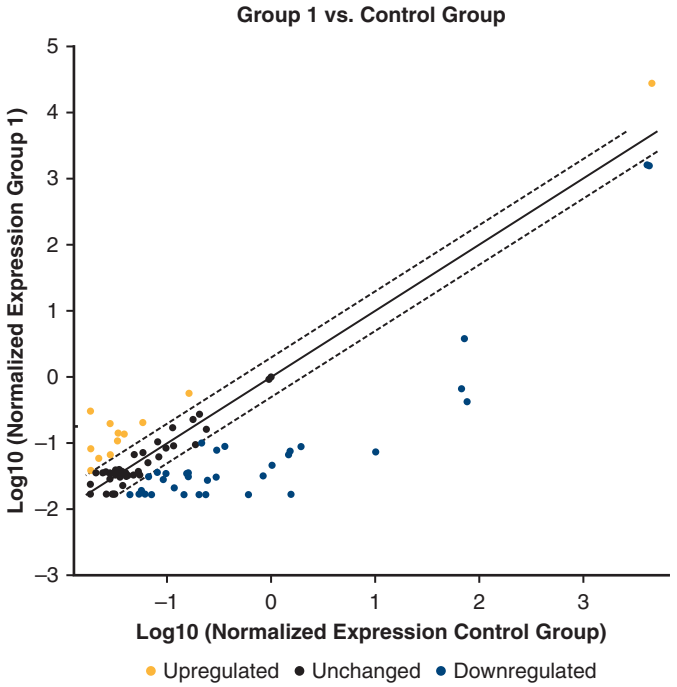


FIGURE 4. Scatter plot from RT^2 Profiler PCR Array for 84 genes related with angiogenesis. *Yellow dots* are genes upregulated, and *blue dots* are genes downregulated in the test group (group 1) compared with the control group. The *central line* indicates unchanged gene expression; the boundaries represent the 2-fold regulation threshold.

insignificantly in the test group. MMPs, especially MMP14 (MT1-MMP), enhance angiogenesis because they have roles in the remodeling of basement membranes and degradation of components of ECM. TIMPs are specific natural inhibitors of MMPs and involved in the degradation of ECM. The balance between MMPs and TIMPs is important in vascular remodeling and angiogenesis.[27] We observed differences between the 2 groups when comparing the gene expression levels of MMPs, TIMPs, and chemokines in PEA specimens. This shows that inflammation has a complicated role in CTEPH development.[28]

Study Limitations

This study has some limitations. First, the sample size of our study was small, which led to nonsignificant results. We aimed to have a bigger sample size for the study, but COVID-19 occurred just after we started to collect samples. Thus, we could not collect more samples because of the lack of supply, shutdown of the hospitals, and diversion of the fundings to the pandemic research projects. Nevertheless, this study might be a guide to the researchers who are focused on the underlying mechanisms of CTEPH. Second, the study needs confirmation of the results. All downregulated and upregulated gene results should be validated with individual PCR assays before going further with new studies. Third, the study has a short follow-up time. We need larger volume studies with longer follow-up time for investigating the correlation between angiogenetic factors and long-term outcomes. The high number of genes is the last limitation. Nevertheless, these initial data will lead to future studies to identify a specific and narrow gene pool for screening CTEPH development in patients with a history of acute pulmonary thromboembolism. In addition, these genes might be useful in detecting patients with CTEPH with a worse prognosis, which may lead to early diagnosis and treatment for better outcomes. In light of these initial data, we pursue a continuation study focusing on the described issues.

CONCLUSIONS

Our findings showed that angiogenic and antiangiogenic factors in patients with CTEPH take roles in a complicated balance. Some of them are correlated with worse hemodynamic measurements of patients with CTEPH. These results help us to develop new biomarkers that might be predictors for adverse outcomes of patients with CTEPH. It is an important step to identify those factors, especially the ones paving the way for severe disease. Application of screening programs with these biomarkers at the early stages of the disease can provide information about the prognosis, and this might lead us to a surgical decision

TABLE 3. Pulmonary endarterectomy specimens from patients with chronic thromboembolic pulmonary hypertension were subjected to quantitative analysis using RT^2 Profiler PCR Array

Gene name	Protein	Fold regulation	*P* value
AKT1	AKT serine/threonine kinase 1	1.04	.418
ANG	Angiogenin	−1.87	.383
ANGPT1	Angiopoietin 1	−1.10	.540
ANGPT2	Angiopoietin 2	2.08	.873
ANGPTL4	Angiopoietin like 4	1.03	.998
ANPEP	Alanyl aminopeptidase, membrane	−1.07	.535
ADGRB1	Adhesion G protein-coupled receptor B1	−1.62	.223
CCL11	C-C motif chemokine ligand 11	−2.14	.422
CCL2	C-C motif chemokine ligand 2	1.25	.679
CDH5	Cadherin 5	4.16	.186
COL18A1	Collagen type XVIII alpha 1 chain	1.32	.500
COL4A3	Collagen type IV alpha 3 chain	1.22	.483
CTGF	Connective tissue growth factor	1.04	.659
CXCL1	C-X-C motif chemokine ligand 1	1.00	.614
CXCL10	C-X-C motif chemokine ligand 10	−5.57	.408
CXCL5	C-X-C motif chemokine ligand 5	−1.75	.329
CXCL6	C-X-C motif chemokine ligand 6	1.21	.676
CXCL9	C-X-C motif chemokine ligand 9p	3.52	.281
EDN1	Endothelin 1	−3.18	.288
EFNA1	Ephrin A1	−3.33	.800
EFNB2	Ephrin B2	−1.10	.540
EGF	Epidermal growth factor	−2.60	.209
ENG	Endoglin	3.19	.181
EPHB4	EPH receptor B4	1.28	.783
ERBB2	Erb-b2 receptor tyrosine kinase 2	1.50	.624
F3	Coagulation factor III, tissue factor	1.09	.423
FGF1	Fibroblast growth factor 1	−1.26	.970
FGF2	Fibroblast growth factor 2	−3.90	.878
FGFR3	Fibroblast growth factor receptor 3	−1.67	.883
FIGF	Vascular endothelial growth factor D	−1.02	.424
FLT-1	VEGFR-1	1.05	.770
FN1	Fibronectin 1	−1.16	.515
HGF	Hepatocyte growth factor	−1.44	.302
HIF1A	Hypoxia inducible factor 1 subunit alpha	2.67	.289
HPSE	Heparanase	−2,90	.177
ID1	Inhibitor of DNA binding 1, HLH protein	−1.14	.354
IFNA1	Interferon alpha 1	−8.98	.424
IFNG	Interferon gamma	−1.45	.332
IGF1	Insulin-like growth factor 1	−4.44	.256
IL1B	Interleukin-1 beta	−2.15	.242
IL6	Interleukin-6	−1.29	.658
CXCL8	C-X-C motif chemokine ligand 8	−1.10	.540
ITGAV	Integrin subunit alpha V	1.70	.902

(Continued)

TABLE 3. Continued

Gene name	Protein	Fold regulation	*P* value
ITGB3	Integrin subunit beta 3	−1.82	.298
JAG1	Jagged 1	−1.09	.651
KDR	Kinase insert domain protein receptor, VEGFR-2	4.54	.243
LECT1	Chondromodulin	−1.10	.540
LEP	Leptin	−1.40	.414
MDK	Midkine	1.28	.577
MMP14	Matrix metallopeptidase 14	−4.26	.059
MMP2	Matrix metallopeptidase 2	1.45	.855
MMP9	Matrix metallopeptidase 9	−2.82	.173
NOS3	Nitric oxide synthase 3	−5.13	.424
NOTCH4	Notch 4	−1.96	.424
NRP1	Neuropilin 1	1.39	.448
NRP2	Neuropilin 2	−1.15	.417
PDGFA	Platelet-derived growth factor subunit A	16.61	.255
PECAM1	Platelet and endothelial cell adhesion molecule 1	−1.10	.540
PF4	Platelet factor 4	1.17	.505
PGF	Placental growth factor	1.28	.660
PLAU	Plasminogen activator, urokinase	2.34	.360
PLG	Plasminogen	−1.34	.803
PROK2	Prokineticin 2	1.45	.678
PTGS1	Prostaglandin-endoperoxide synthase 1	1.23	.598
S1PR1	Sphingosine-1-phosphate receptor 1	−1.32	.905
SERPINE1	Serpin family E member 1	−21.83	.424
SERPINF1	Serpin family F member 1	−21.83	.424
SPHK1	Sphingosine kinase 1	−3.68	.391
TEK	TEK receptor tyrosine kinase	−3.94	.424
TGFA	Transforming growth factor alpha	−137.54	.279
TGFB1	Transforming growth factor beta 1	−92.03	.424
TGFB2	Transforming growth factor beta 2	−20.38	.424
TGFBR1	Transforming growth factor beta receptor 1	−22.74	.424
THBS1	Thrombospondin 1	−26.07	.424
THBS2	Thrombospondin 2	−36.07	.424
TIE1	Tyrosine kinase with immunoglobulin-like and EGF-like domains 1	−1.50	.427
TIMP1	TIMP metallopeptidase inhibitor-1	−9.68	.424
TIMP2	TIMP metallopeptidase inhibitor-2	−14.06	.424
TIMP3	TIMP metallopeptidase inhibitor-3	3.55	.871
TNF	Tumor necrosis factor	−8.61	.424
TYMP	Thymidine phosphorylase	−1.10	.540
VEGFA	Vascular endothelial growth factor A	3.52	.178
VEGFB	Vascular endothelial growth factor B	−2.21	.120
VEGFC	Vascular endothelial growth factor C	−12.37	.389

Shown are 84 angiogenesis-related genes, the proteins that are encoded by them, and the fold changes in the test group compared with the control group.

before deterioration of the patient's condition. Thus, we can create an awareness for screening and building a new algorithm for this specific group of patients. Moreover, those new biomarkers make it possible to screen and detect these patients to provide a precautious treatment plan for better outcomes.

Conflict of Interest Statement

The authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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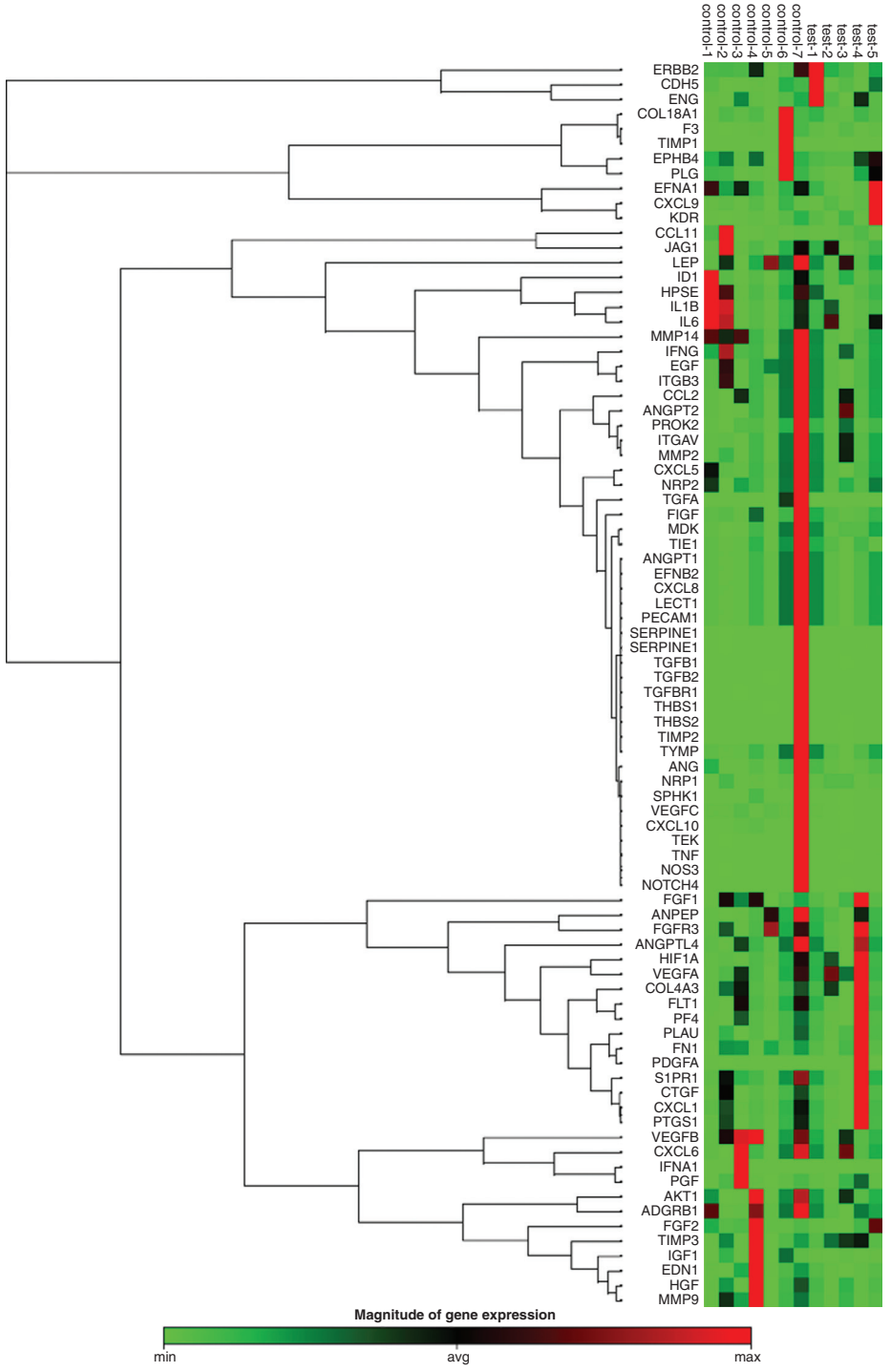

FIGURE E1. Cluster gram from RT^2 Profiler PCR Array performed with the PEA specimens. The heat map shows significant alterations in the expression of 84 genes implicated in angiogenesis. The control group includes the patients whose PVR levels were less than 1000 dynes·sec·cm^{-5}. The test group includes the patients whose PVR levels were more than 1000 dynes·sec·cm^{-5}.

TABLE E1. Data from relevant studies to compare results and outcomes

Study	Purpose	Patient population	Methods	Results	Outcome
Gu and colleagues 2014[15]	The identification of genes associated with CTEPH Provide insight into the pathogenesis of CTEPH and may aid in diagnosis and treatment	Comparison of 5 patients with CTEPH with 5 healthy individuals	Human cDNA of pulmonary artery endothelial cells tested with Human Gene 2.0 ST GeneChip arrays (Affymetrix, Inc)	Alterations in JAK3, GNA15, MAPK13, F2R genes	Potential candidates for distinguishing between CTEPH from healthy individuals in the future
Naito and colleagues 2018[14]	The investigation of the angiogenesis-related characteristics of ECs from a viewpoint of HGF Provide novel diagnostic and therapeutic tools for patients with CTEPH	Comparison of 5 patients with CTEPH and 3 patients with lung cancer	Human pulmonary artery endothelial cells tested with T2-ProfilerTM PCR Arrays (Qiagen)	An angiogenesis-focused gene PCR Array revealed a high expression of HGF in CTEPH endothelial cells.	May provide novel diagnostic and therapeutic tools for patients with CTEPH in the future
Ermerak and colleagues 2022	Identification of angiogenic and antiangiogenic biomarkers Provide novel diagnostic tools for screening and early diagnosis for patients with worse prognosis	Comparison of 3 patients with CTEPH having PVR levels >1000 with 5 patients having PVR levels <1000 dynes·sec·cm^{-5}	Human pulmonary artery tissues tested with Human Angiogenesis RT^2 Profiler PCR Array (Qiagen)	Alterations in TGFA, TGFB1, THBS2, THBS1, TGFBR1, SERPINE1, SERPINF1, TGFB2, TIMP2, VEGFC, IFNA1, TNF, CXCL10, NOS3, IGF1, MMP14, and PDGFA	New biomarkers make it possible to screen and enable precautious treatment management for better outcomes in the future.

Although few studies have investigated the angiogenic and antiangiogenic biomarkers of CTEPH, our study focuses on the biomarkers for comparing samples between high and low PVR levels, which are the most important predictors of the severity and prognosis of the disease. Thus, data may help us to identify the patients with a worse prognosis and to make screening possible for early diagnosis and treatment. *CTEPH*, Chronic thromboembolic pulmonary hypertension; *EC*, endothelial cell; *HGF*, hepatocyte growth factor; *PVR,* pulmonary vascular resistance.