Analytical Cellular Pathology

Advances in Pulmonary Hypertension: Cellular Pathology, Molecular Targets and Therapy

Lead Guest Editor: Kai Yang
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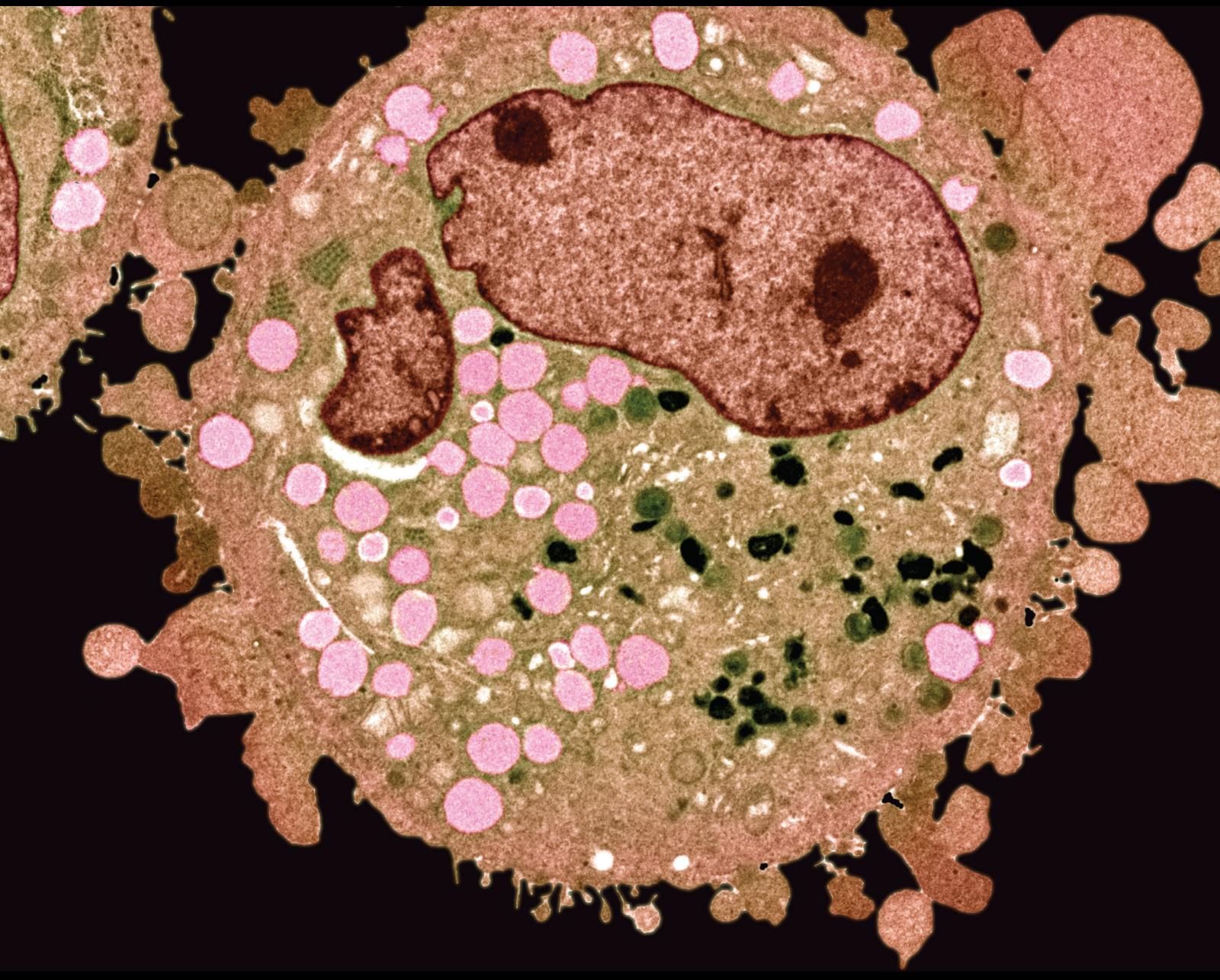

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Hindawi
Analytical Cellular Pathology
Volume 2019, Article ID 3013765, 10 pages
https://doi.org/10.1155/2019/3013765

Research Article

SGK1 Mediates Hypoxic Pulmonary Hypertension through Promoting Macrophage Infiltration and Activation

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Received 8 August 2019; Accepted 25 September 2019; Published 13 November 2019

Guest Editor: Ruifeng Zhang

Inflammation plays a pivotal role in the development of pulmonary arterial hypertension (PAH). Meanwhile, serum glucocorticoid-regulated kinase-1 (SGK1) has been considered to be an important factor in the regulation of inflammation in some vascular disease. However, the role of SGK1 in hypoxia-induced inflammation and PAH is still unknown. WT and SGK1$^{-/-}$ mice were exposed to chronic hypoxia to induce PAH. The quantitative PCR and immunohistochemistry were used to determine the expression of SGK1. The right ventricular hypertrophy index (RVHI), RV/BW ratio, right ventricle systolic pressure (RVSP), and percentage of muscularised vessels and medical wall thickness were measured to evaluate PAH development. The infiltration of macrophages and localization of SGK1 on cells were examined by histological analysis. The effects of SGK1 on macrophage function and cytokine expression were assessed by comparing WT and SGK1$^{-/-}$ macrophages in vitro. SGK1 has high expression in hypoxia-induced PAH. Deficiency of SGK1 prevented the development of hypoxia-induced PAH and inhibited macrophage infiltration in the lung. In addition, SGK1 knockout inhibited the expression of proinflammatory cytokines in macrophages. SGK1-induced macrophage activation and proinflammatory response contributes to the development of PAH in hypoxia-treated mice. Thus, SGK1 might be considered a promising target for PAH treatment.

1. Introduction

Pulmonary arterial hypertension (PAH) is a progressive and life-threatening disease with a poor prognosis [1]. PAH is characterized by pulmonary vasoconstriction and increased pulmonary vascular resistance leading to right ventricular failure, fluid overload, and death [2]. The major histopathological feature of PAH is vascular wall remodeling, and the remodeling process includes intima proliferation, the medial and adventitial layer hypertrophy, and extracellular matrix deposition [3]. Pulmonary arteries exhibit complex structural and functional changes in PAH, and various cell types and growth factors are involved in the development of PAH [4]. Although PAH is primarily considered to be a vascular disease, there is a well-established link between PAH and inflammation [5], and evidence from many clinical and basic researches suggests that inflammation plays an important role in the process of PAH [6].

Pulmonary vascular lesions in patients with PAH and the animal models of pulmonary hypertension are characterized by infiltration of many inflammatory cells, including T lymphocytes, B lymphocytes, macrophage, dendritic cells, and mast cells, around the blood vessels [6]. Among these inflammatory cells in PAH, the monocytes/macrophages are more often associated with the disease [7, 8]. CD68^{+} macrophages are observed in advanced obliterative plexiform lesions in both experimental and clinical PAH [8–10]. Depletion or inactivation of macrophages may inhibit PAH in a variety of model systems, including experimentally induced hypoxic PAH and portopulmonary hypertension [11, 12]. The

activation of macrophages promotes vascular injury and the development of angioobliterative pulmonary hypertension by inducing pulmonary artery endothelial cell injury and apoptosis as well as smooth muscle cell proliferation [12]. It is still not clear which key factors regulate macrophage activation and ultimately promote PAH development.

Serum glucocorticoid-regulated kinase 1 (SGK1) belongs to the cAMP-dependent protein kinase 1/cGMP-dependent protein kinase/protein kinase C family [13]. The SGK1 promoter contains a number of transcription factor binding sites that are responsible for the stimulated regulation of SGK1 expression [14]. Because of these binding sites, SGK1 is primarily transactivated in response to various hormonal and nonhormonal extracellular stimuli [15]. Under physiological conditions, the majority of cells express low levels of SGK1, and the expression of SGK1 in some cells is much higher under certain pathophysiological conditions. Tissue ischemia, tissue hypoxia, and tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) and other inflammatory stimuli induce high SGK1 expression [14, 16–18]. SGK1 participates in various biological activities such as cell proliferation, cell apoptosis, and ion channel regulation, and it plays an important role in the regulation of inflammation [14, 19–21]. SGK1 is considered to be an important factor regulating inflammation and vascular disease development [22–24]. However, the role of SGK1 in hypoxia-induced PAH remains unclear.

In this study, we investigated the role of SGK1 in response to chronic hypoxia. Our results indicate that deficiency of SGK1 attenuates hypoxia-induced pulmonary hypertension. This attenuation was associated with inhibition of vascular remodeling and reduction in the inflammatory response. Additionally, we show that SGK1 deficiency inhibits the activation of macrophages in vitro. These data provide first proof to describe an important role of SGK1 in macrophage activation and hypoxia-induced PAH development.

2. Materials and Methods

2.1. Mouse Models. SGK1 knockout ($SGK1^{-/-}$) and littermate control (WT) mice were generated as before [25]. At the age of 10–12 weeks, male $SGK1^{-/-}$ mice and their WT littermates had free access to food and were housed under specific pathogen-free conditions with a 12 : 12 hour light–dark cycle between 20 and 24°C. WT and $SGK1^{-/-}$ mice were maintained with standard laboratory chow and water ad libitum. All animal housing and experimental protocols were approved by the Animal Care and Use Committee of Capital Medical University and conformed to the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978).

2.2. Exposure to Chronic Hypoxia. WT and $SGK1^{-/-}$ mice were randomly assigned to the normoxia group (N) and hypoxia group (H). The normoxia group was exposed to room air, whereas the hypoxia-group mice were placed in a ventilated chamber with 10% O_2 and $CO_2 < 0.5\%$ environment. The environment was established by a mixture of room air and nitrogen with a detector (RCI Hudson, Anaheim, CA, USA) which monitored and controlled the fractional concentration of O_2 automatically. The chamber was opened every 3 to 4 days for changing cage and replenishing food and water.

2.3. Hemodynamics and Ventricular Weight Measurements. After 4 weeks of normoxia or hypoxic exposure, right ventricular systolic pressure (RVSP) and right ventricular hypertrophy index (RVHI) was measured as previously described [26]. Briefly, right ventricular pressure (RVP) was measured with a pressure transducer catheter and AcqKnowledge software (Biopac Systems Inc., CA, USA) by a 23-gauge needle via the diaphragm into the right ventricle (RV). The RVSP was then recorded as a surrogate for pulmonary artery pressure. Mouse hearts were excised and right ventricular hypertrophy was evaluated by right ventricular hypertrophy index (RVHI), which was the wet weight ratio of the right ventricle (RV) to left ventricle (LV) plus septum (S). The RV/BW ratio was obtained through measuring the right ventricular mass and body weight.

2.4. Histological Analysis. After anesthesia, the mice were perfused with PBS through LV. Then, the lung tissues of mice were harvested, fixed in formalin, and embedded in paraffin. Lung cross sections at 5 μm intervals were stained by hematoxylin and eosin (H&E) with standard procedures. For immunohistochemistry, lung sections were incubated with antibodies against SGK1 (1 : 100, Abcam, Cambridge, MA), α-smooth muscle actin (α-SMA, 1 : 100, ZSGB-BIO, Beijing, China), or Mac3 (1 : 200, Santa Cruz Biotechnology, Dallas, TX) at 4°C overnight, then, with secondary antibodies at 4°C for 1 hour and detected with 3,3-diaminobenzidine, and sections were counterstained with hematoxylin.

Morphometric analysis to quantify pulmonary arterial (PA) remodeling was made on the distal small PA by measuring PA wall thickness or the thickness of the smooth muscle layer in H&E and α-SMA stain images of lung cross sections captured by a Nikon Eclipse Ni microscope (Nikon, Tokyo, Japan) and analyzed by NIS-Elements AR 4.0 software (Nikon). Muscularised vessel % was calculated as the number of muscularised vessels/total number of vessels counted per section $\times$ 100 and medial wall thickness % (MWT%) (the ratio of the average medial thickness divided by the average vessel radius) was assessed in 30 muscular PA with an external diameter of 50–100 μm per lung section. Mac-3-positive cells per vessel were determined by counting 30 to 35 vessels per mouse. Mac-3-positive cells per 30 HPF were counted in 30 high-power fields (magnification: 400x) per mouse.

For immunofluorescence, lung sections or cells were labeled with primary antibodies F4/80 (1 : 100, Abcam) and SGK1 (1 : 100, Abcam) and then incubated with Alexa Fluor 555- and 488- conjugated secondary antibody (1 : 500, Thermo Fisher). Images were captured by a Leica TSC-SP5 laser-scanning confocal microscope (Leica, Wetzlar, Germany).

2.5. Macrophage Culture. Bone marrow-derived macrophages were prepared with a modification as described previously [27]. In brief, bone marrow cells were obtained by flushing from the dissected femurs and tibias, and cells were resuspended and cultured in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 10% FBS in

the presence of 50 ng/ml recombinant murine M-CSF. Macrophages were stimulated with 50 ng/ml LPS (Sigma-Aldrich, St. Louis, MO) for 4 h.

2.6. Quantitative RT-PCR Analysis. Bone marrow-derived macrophage RNA was extracted by TRIzol reagent according to the manufacturer's protocol (Thermo Fisher). Equal amounts of RNA (1 μg) were added to reverse transcriptase reaction mix with oligo-dT primers (Promega, Southampton, UK). SYBR Premix Ex Taq (TaKaRa, Shiga, Japan) was used to perform quantitative real-time PCRs with IQ5 Multicolor Real-Time PCR Detection System (Bio-Rad, Hercules, CA). The following primers were used: SGK1 (5′-CGTTCGGTC TTCATTCAC-3′ forward; 5′-TTATAGATCCAGGGTG GC-3′ reverse), MCP-1 (5′-CTGAAGCCAGCTCTCTCTT CCT-3′ forward; 5′-CAGGCCCAGAAGCATGACA-3′ reverse), TNF-α (5′-CACAAGATGCTGGGACAG-TGA-3′ forward; 5′-TCCTTGATGGTGGTGCATGA-3′ reverse), IL-1β (5′-CCATGG-CACATTCTGTTCAAA-3′ forward; 5′-GCCCATCAGAGGCAAGGA-3′ reverse), IL-10 (5′ -CCAGGGAGATCCTTTGATGA-3′ forward; 5′-CATT-CCCAGAGGAATTGCAT-3′ reverse), and GAPDH (5′ -CATGGCCTTCCGTGTTCCTA-3′ forward; 5′-GCGGCA CGTCAGATCCA-3′ reverse).

2.7. Cytometric Bead Array (CBA). To measure the release of cytokines (IFN-γ, IL-6, IL-12, MCP-1, TNF, and IL-10), we used the BD CBA mouse inflammation kit (BD Biosciences, San Jose, CA). The macrophage culture supernatant was collected 4 hours after LPS stimuli, and the assays were performed according to the manufacturer's instruction. The results were analyzed with FCAP Array Version 3.0 (Soft Flow, Duesseldorf, Germany).

2.8. Statistical Analysis. All data are expressed as mean ± SD. Statistics were calculated with SPSS computer software for Windows (version 13.0; SPSS, Chicago, IL). The Student *t*-test was used to compare data between two groups in each experiment. One-way ANOVA, followed by a Bonferroni–Dunn test, was used to compare data between three or more groups in each experiment. Results were considered to represent significant differences at $p < 0.05$.

3. Results

3.1. Hypoxia-Induced PAH Increases SGK1 Expression in Mouse Lung. To investigate the role of SGK1 in the development of chronic PAH, we examined SGK1 expression in the lungs of mouse with hypoxia. The mRNA level of SGK1 was upregulated in the lungs of hypoxia-induced mice compared to the lungs of normoxia-induced mice (Figure 1(a)). Immunohistochemistry revealed that SGK1-positive cells had infiltrated into the alveolar space of the lungs in hypoxia-induced mice (Figure 1(b)). Thus, SGK1 expression and activation may play an important role in the development of hypoxia-induced PAH.

3.2. SGK1 Deficiency Inhibits Hypoxia-Induced PAH Development. Given that SGK1 was upregulated in the lungs of hypoxia-induced mice, we sought to determine the effect of SGK1 on the development of PAH. Both WT- and SGK1-deficient ($SGK1^{-/-}$) mice were exposed to hypoxia or normoxia for 4 weeks. Hypoxia treatment markedly increased RV/(LV+S) and RV/BW ratios in both WT and $SGK1^{-/-}$ mice compared to normoxia-treated mice, while $SGK1^{-/-}$ mice significantly reduced RV/(LV+S) and RV/BW ratios compared to WT mice under hypoxic conditions (Figures 2(a) and 2(b)). RVSP was much higher in hypoxic WT and $SGK1^{-/-}$ mice than in normoxic WT and $SGK^{-/-}$ mice. However, this increase in RVSP was inhibited in $SGK1^{-/-}$ mice compared to WT mice under hypoxic conditions (Figures 2(c) and 2(d)). These results suggest that SGK1 aggravates right ventricular systolic pressure and right ventricular hypertrophy in hypoxia-induced PAH.

3.3. SGK1 Deficiency Reduces Pulmonary Arterial Remodeling in Hypoxia-Induced PAH. To determine the effects of SGK1 on pulmonary arterial remodeling, we investigated the percentage of muscularised vessels (the number of muscularised vessels/total number of vessels) by H&E staining and the percentage of medial wall thickness (MWT%, the ratio of the average medial thickness divided by the average vessel radius) by immunohistochemical staining of α-SMA. Under hypoxia, the muscularised vessels were increased in WT and $SGK1^{-/-}$ mice, while $SGK1^{-/-}$ mice significantly decreased muscularised vessels compared to WT mice (Figures 3(a) and 3(b)). The pulmonary artery wall area to total area ratio was significantly higher in the PAH model groups compared with the control groups, while SGK1 deficiency significantly reduced the ratio of the wall area to total area (Figures 3(c) and 3(d)). Therefore, SGK1 promotes pulmonary arterial remodeling in hypoxia-induced PAH.

3.4. SGK1 Deficiency Inhibits Inflammatory Response in Hypoxia-Induced PAH. Previous studies have shown that inflammation exacerbates pulmonary vascular remodeling during PAH [28] and depletion or inactivation of macrophages can inhibit PAH development [11, 12]. For these reasons, the effect of SGK1 on regulating pulmonary inflammation was investigated by detecting macrophage infiltration. Macrophage infiltration was detected by immunohistochemical staining of Mac3 (macrophage marker), and macrophage accumulation was quantitated by counting the number of macrophages around the blood vessels and the overall number of macrophages in the lung. Under hypoxia, the number of macrophages around the blood vessels was increased in WT and $SGK1^{-/-}$ mice, while $SGK1^{-/-}$ mice significantly decreased the number of macrophages around the blood vessels compared to WT mice (Figures 4(a) and 4(b)). SGK1 deficiency also significantly inhibited macrophage infiltration in the lung under hypoxic conditions (Figures 4(c) and 4(d)). Thus, SGK1 promotes macrophage infiltration in hypoxia-induced PAH.

3.5. SGK1 Expression on Macrophage Is Essential for PAH Development. We detected the localization of SGK1 in hypoxia-induced lungs by double immunofluorescence

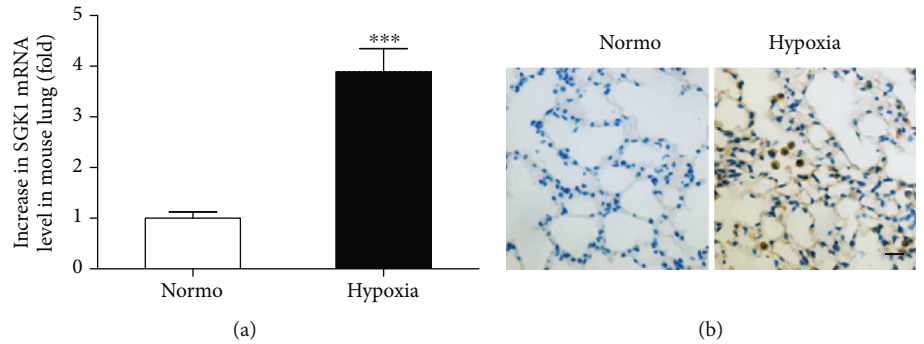

Figure 1: Hypoxia-induced PAH increased expression of SGK1 in mice. (a) Lung mRNA levels for SGK1 were measured using quantitative real-time PCR from mice exposed to 3 days of normoxia or hypoxia. GAPDH was used to normalize the quantitative real-time data. Results are expressed as relative fold changes compared with the normoxia group ($n = 6 - 7$ in each group). (b) Immunohistochemical analysis of SGK1 expression in lung sections from mice exposed to 7 days of normoxia or hypoxia. Scale bar = 10 μm. $^{***}p < 0.001$.

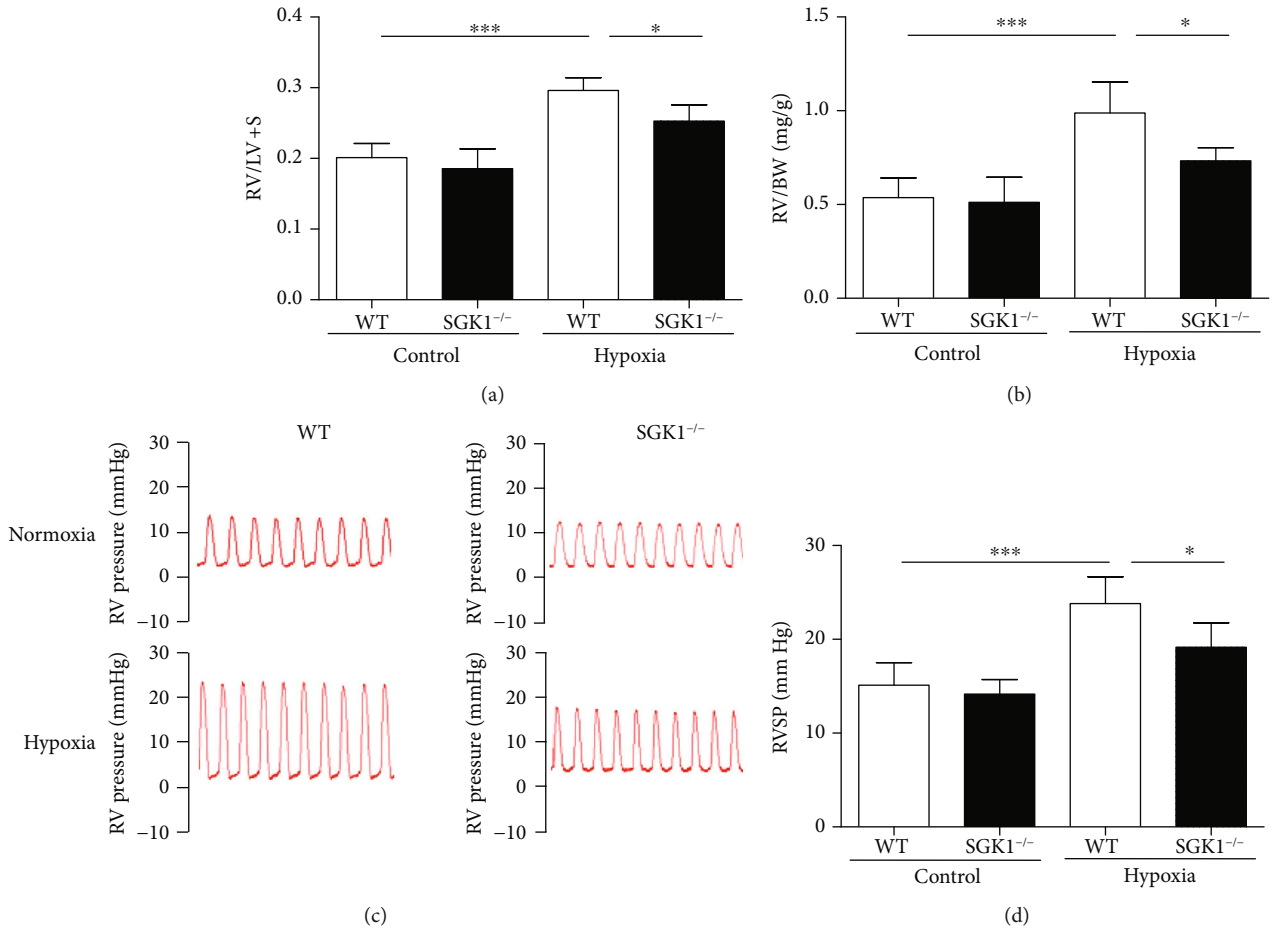

Figure 2: SGK1 deficiency alleviated the development of PAH in mice. (a) Graphs of right ventricular hypertrophy index (right ventricle/left ventricle plus septum weight, RV/(LV+S)) from mice exposed to 28 days of normoxia or hypoxia. (b) Graphs of right ventricular mass to body weight ratio (RV/BW) from mice exposed to 28 days of normoxia or hypoxia. (c) Representative images of right ventricular systolic pressure (RVSP) waves (red) from WT and SGK1$^{-/-}$ mice exposed to 28 days of normoxia or hypoxia. (d) Graphs of right ventricular systolic pressure (RVSP). $n = 6$ in each group, $^{*}p < 0.05$, $^{***}p < 0.001$.

staining. The results showed that SGK1 mainly colocalize with F4/80, a marker of macrophages (Figure 5(a)). We isolated macrophages from bone marrow and detected SGK1 expression by immunofluorescence staining and found SGK1 and F4/80 were colocalized in bone marrow-derived macrophages (Figure 5(b)). Thus, SGK1 is mainly expressed on macrophages in hypoxia-induced lungs and macrophages isolating from the bone marrow.

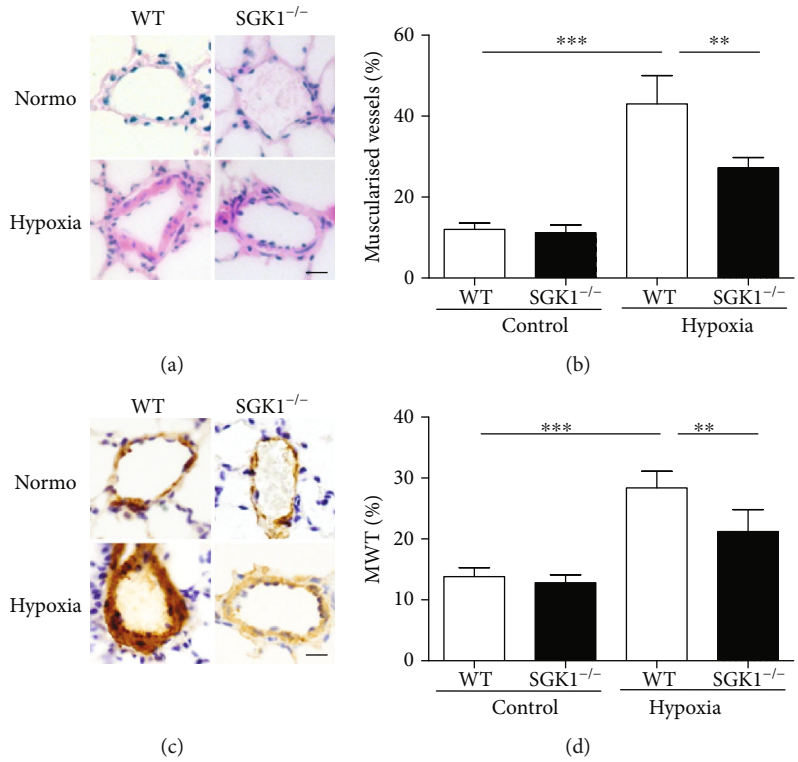

Figure 3: SGK1 deficiency alleviated pulmonary arterial remodeling of PAH in mice. (a) Representative H&E images of pulmonary arteries from mice exposed to 28 days of normoxia or hypoxia. (b) Graphs of muscularised vessels calculated as the number of muscularised vessels to total number of vessels. (c) Representative immunohistochemical staining of α-SMA images in pulmonary arteries from mice exposed to 28 days of normoxia or hypoxia. (d) Graphs of medial wall thickness calculated as the average medial thickness to the average vessel radius. Scale bar = 10 μm. $n = 6$ in each group, $^{**}p < 0.01$, $^{***}p < 0.001$.

3.6. SGK1 Promotes Macrophage Activation and Inflammatory Response In Vitro. We next explored the mechanisms of inhibiting pulmonary inflammatory response in SGK1$^{-/-}$ mice with PAH. As SGK1 was mainly expressed on macrophages and macrophages were involved in the process of inflammation and tissue injury by secreting numerous biologically active molecules, we detected the secretion of cytokines in SGK1$^{-/-}$ macrophages with LPS stimulation. Cytokine expressions of WT and SGK1$^{-/-}$ macrophages derived from bone marrow with LPS stimulation were analyzed by quantitative PCR. As shown in Figure 6, LPS significantly increased the expression of MCP-1, IL-1β, TNF-α, and IL-10 from cultured WT macrophages, and SGK1 knockout reduced the expression of proinflammatory cytokines, MCP-1, IL-1β, and TNF-α. Meanwhile, SGK1 knockout had no significant effect on the expression of anti-inflammatory cytokine, IL-10 (Figure 6(d)). The concentrations of cytokines in the supernatant of WT and SGK1$^{-/-}$ macrophages with LPS stimulation were analyzed by BD cytometric bead array (CBA) with a mouse inflammation kit. Consistent with the results of mRNA expression, SGK1 knockout inhibited the production of proinflammatory cytokines, IFN-γ, IL-6, IL-12, MCP-1, and TNF, and had no significant effect on the production of anti-inflammatory cytokine, IL-10 (Figure 7). Therefore, SGK1 promotes macrophage activation and proinflammatory response in vitro.

4. Discussion

We provide the first evidence for the critical role of SGK1 in hypoxia-induced PAH by regulating proinflammation response. Hypoxia-induced PAH significantly increased the expression of SGK1 in mouse lung. Lack of SGK1 markedly ameliorated hypoxia-induced PAH development and pulmonary arterial remodeling. SGK1 deficiency also inhibited inflammatory response in PAH. Moreover, SGK1 is expressed on macrophages in hypoxia-induced lungs in vivo, and SGK1 deletion in bone marrow-derived macrophages inhibited secretion of proinflammatory cytokines in vitro. Our findings suggest that SGK1 plays an important role in PAH development and may be a promising therapeutic target for PAH therapy.

Previous studies have shown that perivascular immunity and inflammation play a key role in the pathogenesis of idiopathic PAH [29]. Infiltrates of inflammatory cells, including macrophages, dendritic cells, and T and B cells, have been detected in pulmonary vascular lesions of patients with severe pulmonary hypertension [28]. And leukocytes and inflammatory mediators have been detected in experimental PAH models such as monocrotaline-treated rats [9, 30] and hypoxia-induced mice [8, 31]. In addition to infiltration of pulmonary immune cells, elevated levels of serum inflammatory cytokines and chemokines have been reported in patients with PAH [32], and some elevated levels of

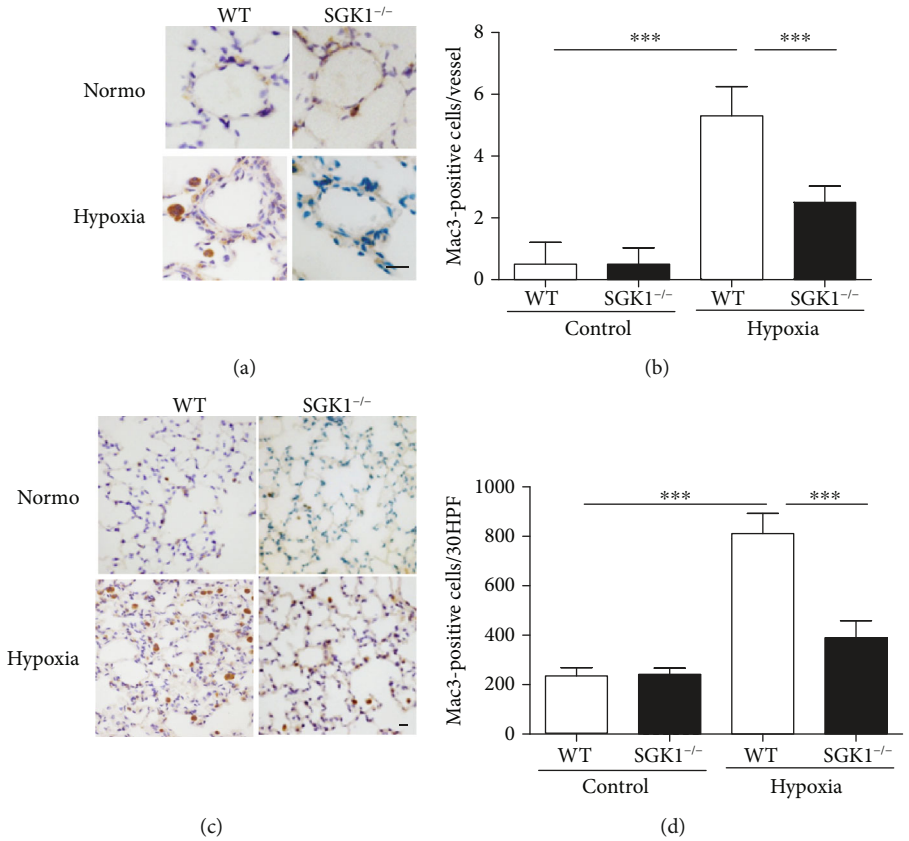

Figure 4: SGK1 deficiency decreased macrophage accumulation in hypoxic mice. (a) Representative immunohistochemical staining of Mac3 images in pulmonary arteries from mice exposed to 28 days of normoxia or hypoxia. (b) Graphs of Mac3-positive cells per vessel. (c) Representative immunohistochemical staining of Mac3 images in the lungs from mice exposed to 28 days of normoxia or hypoxia. (d) Graphs of Mac3-positive cells per 30 HPF. Scale bar = 10 μm. $n = 6$ in each group, $^{***}p < 0.001$.

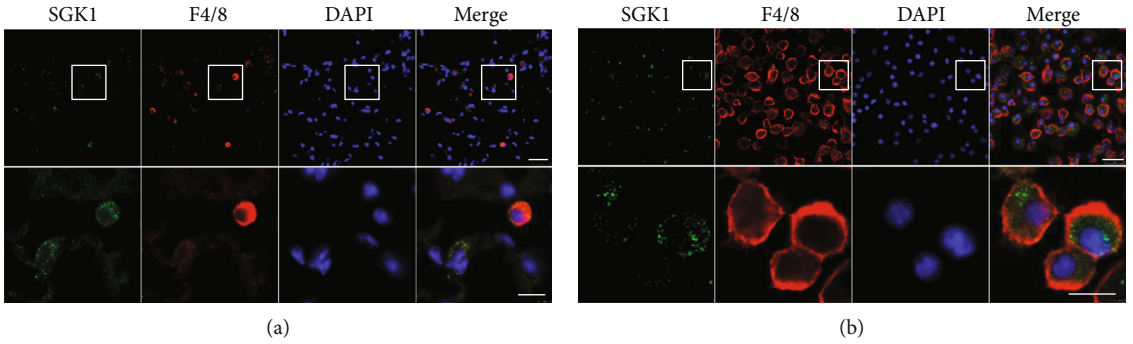

Figure 5: SGK1 expressed on macrophages in hypoxia-induced lungs and macrophages derived from the bone marrow. (a) Representative of lung tissue from WT mice exposed to 7 days of hypoxia. Top panels present the low-magnification images of SGK1 (green), F4/80 (red), and DAPI (blue). Scale bar = 25 μm. Bottom panels present the high-magnification insets from the white boxes of the top panels. Scale bar = 10 μm. (b) Representative of bone marrow-derived macrophage. Top panels present the low-magnification images of SGK1 (green), F4/80 (red), and DAPI (blue). Scale bar = 25 μm. Bottom panels present the high-magnification insets from the white boxes of the top panels. Scale bar = 10 μm.

inflammatory cytokines, such as IL-1β and IL-6, have been shown to predict survival in idiopathic and familial pulmonary arterial hypertension [33]. Previous studies have demonstrated that inflammation may contribute to the development of PAH, and anti-inflammatory treatment may be a promising strategy for severe PAH [34]. Activated $CD68^+$ macrophages accumulated in the pulmonary arteries of hepatopulmonary syndrome and macrophage depletion by intravenous injections of either gadolinium chloride or liposomal clodronate prevented and reversed hepatopulmonary syndrome [11]. In a rat model of severe pulmonary hypertension, macrophages accumulated around pulmonary

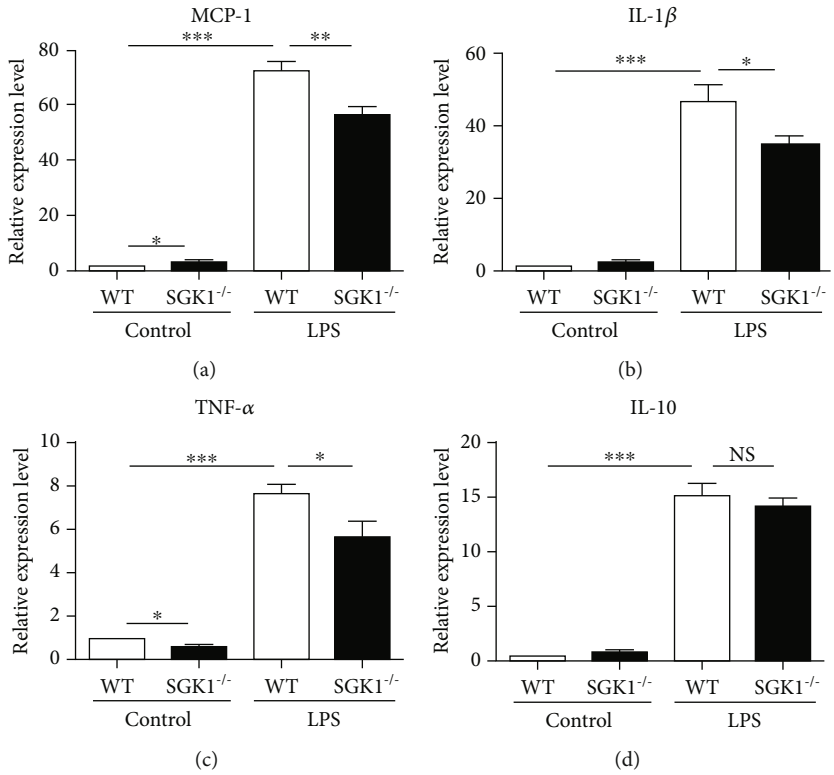

Figure 6: SGK1 deficiency decreased proinflammatory cytokine expression in vitro. Expression of MCP-1 (a), IL-1β (b), TNF-α (c), and IL-10 (d) was analyzed by quantitative PCR in bone marrow-derived macrophage stimulated with 50 ng/ml LPS for 4 hours. GAPDH was used to normalize the quantitative real-time data. Results are expressed as relative fold changes compared with the control group. Data represent at least three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

arterioles, and depleting CD68^{+} macrophages prevented the development of pulmonary hypertension [28]. Thus, depletion or inactivation of macrophages may have a therapeutic potential in pulmonary hypertension. In our study, hypoxia stimulation induced macrophage infiltration in the lung of WT mice, and deficiency of SGK1 inhibited macrophage infiltration. Therefore, SGK1 deletion inhibited the development of PAH and was likely related to suppressed macrophage infiltration. Moreover, deficiency of SGK1 suppressed the activation of macrophages by inhibiting the macrophage expression of proinflammatory cytokines. Our results uncover a possible role for SGK1 in PAH pathogenesis and identify a key molecular that may be amenable to therapeutic targeting.

SGK1, a downstream effector of the phosphoinositide-3 kinase cascade, is a serine-threonine kinase and can be activated by various stimuli, such as steroids and peptide hormones like mineralocorticoids, growth factors like TGF-β, and cytokines like IL-6 [16, 35]. The expression of SGK1 is highly variable and subject to regulation by a wide variety of triggers. SGK1 stimulates various renal tubular ion channels and transporters and is therefore involved in the regulation of renal electrolyte excretion [35]. SGK1 plays an important role in inflammatory responses in cardiac fibroblasts triggered by mechanical stretch [36]. SGK1 promotes survival, invasiveness, motility, epithelial to mesenchymal transition, and adhesiveness of tumor cells [37]. SGK1 contributes to monocyte/macrophage migration and MMP-9 transcription during atherogenesis [23]. In our study, we have found SGK1 expressed on macrophage after pulmonary arterial hypertension (Figure 5(a)), and lack of SGK1 markedly inhibited inflammatory response and ameliorated hypoxia-induced PAH. Our findings suggest that SGK1 expressed on macrophages plays an important role in PAH development.

Previous studies have demonstrated that SGK1 as an important factor for regulating inflammation and vascular disease development [22–24]. In cardiac ischemia-reperfusion injury, SGK1 promotes the release of proinflammatory factors and reduces the level of anti-inflammatory factors [22]. In other vascular diseases, SGK1 also have effects on the inflammation of the disease by regulating macrophage activity [23, 24]. Despite the wide tissue distribution of SGK1 and its sensitivity to various stimuli, its role in hypoxia-induced pulmonary hypertension was not fully defined. In our study, we found SGK1 induced in hypoxia-induced pulmonary hypertension and SGK1 deletion in mice inhibited the development of pulmonary hypertension. Previous studies have demonstrated that increased expression of SGK1 is associated with inflammation. SGK1 played a key role in the induction of pathogenic Th17 cells and facilitated tissue inflammation [20]. SGK1 inhibition reduced proinflammatory cytokine IL-17 and reduced anti-inflammatory cytokines IL-10 and IL-27 in the ischemic-reperfused heart [22]. In a transgenic zebrafish model, SGK1 gene disruption delayed inflammation resolution, without interrupting

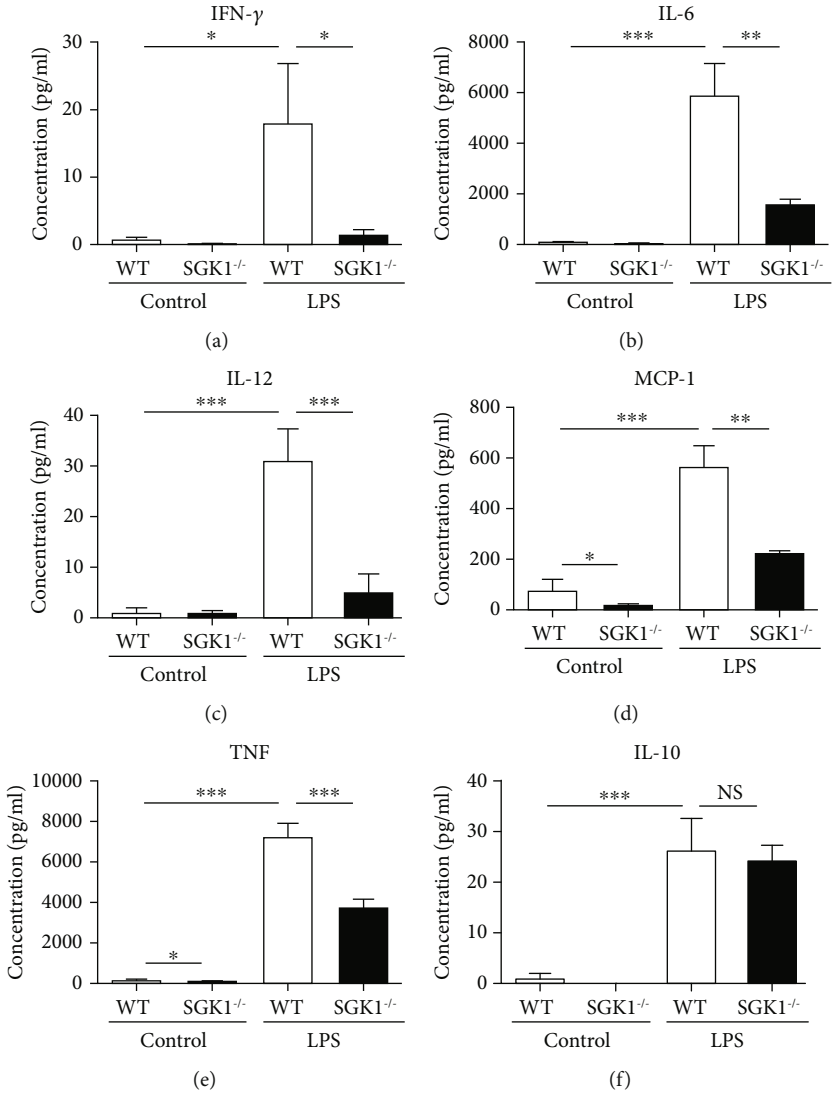

Figure 7: SGK1 deficiency decreased proinflammatory cytokine secretion in vitro. Secretion of IFN-γ (a), IL-6 (b), IL-12 (c), MCP-1 (d), TNF (e), and IL-10 (f) was analyzed by cytometric bead array (CBA) in bone marrow-derived macrophage stimulated with 50 ng/ml LPS for 4 hours. Data represent at least three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

neutrophil infiltration in vivo [21]. These results are consistent with our results in the model of hypoxia-induced pulmonary inflammation, where we showed that the macrophage infiltration was significantly decreased in pulmonary tissue of SGK1 knockout mice compared to that of WT mice. And our findings also suggest an important role of SGK1 in the regulation of pulmonary inflammation and PAH development.

SGK1 can phosphorylate the cAMP-responsive element-binding protein (CREB) and interfere with CREB-dependent gene transcription [38]. SGK1 also could enhance the activity of NFκB. On the one hand, SGK1 activates the kinase IκB kinase beta (IKKβ), which subsequently phosphorylates and degrades IκB, an inhibitor of NFκB, and promotes NFκB entry into the nucleus and turns on gene transcription [14, 39]. On the other hand, SGK1 phosphorylates and activates NDRG1, which is specific target of SGK1. NDRG1 could downregulate NFκB signaling [40]. For NFκB regulating the expression of various cytokines, SGK1 could regulate cytokine expression by activating NFκB. Thus, in our study, SGK1 may promote cytokine expression by activating NFκB.

Macrophages respond to environmental signals with plasticity and undergo different forms of polarized activation, which can be broadly divided into classically activated (M1) macrophages and alternatively activated (M2) macrophages. IFN-γ generated by Th1 cells activates classical macrophages expressing proinflammatory cytokines TNF-α, IL-1β, and IL-6. Th2 cytokines, IL-4, and/or IL-13 activate alternative macrophages expressing IL-10 [41]. In our study, we found that SGK1 deletion inhibited the expression of proinflammatory cytokines, such as IL-6, TNF-α, and IL-1β. Circulating levels of these cytokines, IL-6, TNF-α, and IL-1β, were abnormally elevated in patients with PAH, and all of these cytokines were associated with the poor clinical outcomes in PAH patients [33, 42]. Moreover, both IL-6-overexpressing mice and TNF-α-overexpressing mice

induced spontaneously pulmonary hypertension and pulmonary vascular remodeling [43, 44], while IL-6 knockout inhibited the development of pulmonary hypertension induced by chronic hypoxia [45]. Inhibition of the IL-1 signaling pathway by treatment of IL-1 receptor antagonist reduced pulmonary hypertension in rat [46]. These previous studies suggest that IL-6, TNF-α, and IL-1β may be actively involved in pulmonary hypertension. Thus, in our results, the inhibitory effect of SGK1 knockdown on PAH is likely through suppressing the expression of crucial cytokines, such as IL-6, TNF-α, and IL-1β.

5. Conclusion

In summary, we provide the evidences that SGK1 promotes the development of PAH induced by chronic hypoxia. SGK1 also plays an important role in the inflammation of hypoxia-induced PAH, and deletion of SGK1 is found to attenuate inflammation-associated PAH. Our findings highlight a potential option for therapeutic intervention of PAH.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Xin Xi and Jing Zhang contributed equally to this study.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81500037, 81672151, and 81500365) and Beijing Natural Science Foundation (7172040).

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Hindawi
Analytical Cellular Pathology
Volume 2019, Article ID 5189165, 9 pages
https://doi.org/10.1155/2019/5189165

Research Article

Predictive Value of Novel Inflammation-Based Biomarkers for Pulmonary Hypertension in the Acute Exacerbation of Chronic Obstructive Pulmonary Disease

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Received 3 June 2019; Accepted 6 September 2019; Published 14 October 2019

Guest Editor: Xin Yun

Recently, there has been an increasing interest in the potential clinical use of several inflammatory indexes, namely, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic-immune-inflammation index (SII). This study aimed at assessing whether these markers could be early indicators of pulmonary hypertension (PH) in patients with acute exacerbation of chronic obstructive pulmonary disease (AECOPD). A total of 185 patients were enrolled in our retrospective study from January 2017 to January 2019. Receiver operating characteristic curve (ROC) and area under the curve (AUC) were used to evaluate the clinical significance of these biomarkers to predict PH in patients with AECOPD. According to the diagnostic criterion for PH by Doppler echocardiography, the patients were stratified into two groups. The study group consisted of 101 patients complicated with PH, and the control group had 84 patients. The NLR, PLR, and SII values of the PH group were significantly higher than those of the AECOPD one ($p < 0.05$). The blood biomarker levels were positively correlated with NT-proBNP levels, while they had no significant correlation with the estimated pulmonary arterial systolic pressure (PASP) other than PLR. NLR, PLR, and SII values were all associated with PH ($p < 0.05$) in the univariate analysis, but not in the multivariate analysis. The AUC of NLR used for predicting PH was 0.701 and was higher than PLR and SII. Using 4.659 as the cut-off value of NLR, the sensitivity was 81.2%, and the specificity was 59.5%. In conclusion, these simple markers may be useful in the prediction of PH in patients with AECOPD.

1. Introduction

Chronic obstructive pulmonary disease (COPD), characterized by an incompletely reversible airflow limitation, is not just a chronic inflammatory response involving the airways but a systemic chronic inflammatory syndrome. It is a worldwide health-care burden which poses a significant public health challenge [1]. The Global Burden of Disease Study estimated that there were 174.5 million prevalent COPD patients worldwide in 2015 [2], and COPD will represent the third leading cause of death globally by 2030 [3]. AECOPD indicates a prolonged (≥48 h) worsening of a patient's clinical respiratory manifestations that require additional medications or are severe enough to warrant hospital admission [4]. It is a complex and life-threatening condition which is responsible for a growing mortality, a large proportion of health-care expenditure, an increased risk of dying, and the development of complications in the progression of the disease [5].

Pulmonary hypertension (PH) is a severe and poor prognosis complication of COPD. Although the primary disease progresses slowly, once combined with PH the symptoms aggravate, mortality surges, and the risk of AECOPD increases. COPD patients with PH have a poor long-term prognosis with a median postdiagnosis survival of only 2 to 5 years [6]. Early diagnosis and timely treatment are particularly important in the course of disease progression in our clinical work. The detection methods for PH are mainly divided into invasive and noninvasive examinations. Although right heart catheterization is the "gold standard"

for the diagnosis of PH, it is relatively complicated, expensive, and invasive. As a result, Doppler echocardiography is recommended by the ESC/ERS Guidelines as the primary noninvasive diagnostic instrument in suspected pulmonary arterial hypertension (PAH) in COPD patients [7].

However, the prediction of PH appears to be an impossible mission especially in some community hospitals with inferior methods of examination. Thus, a growing number of researchers are extensively focusing on finding a noninvasive and more easily obtainable biomarker that enables stratification of PH in COPD patients. Recently, NLR, PLR, or SII have been associated with inflammation-linked diseases (malignancy [8], ulcerative colitis [9], and ANCA-associated vasculitis [10], for example). However, as far as we know, few studies have evaluated the utility of these blood-based molecules as predictive biomarkers of PH in AECOPD patients. This article will summarize the predictive significance of these various inflammatory indices and estimate the independent risk factors correlated with PH.

2. Methods

2.1. Study Population. Patients diagnosed with AECOPD ($n = 185$) were registered in this retrospective study. All patients evaluated for PH in our study underwent Doppler echocardiography and were divided into study and control groups depending on whether they also had PH. 101 AECOPD patients with PH were included in the study group, and the remaining eighty-four patients were assigned to the control group.

The inclusion criteria are as follows: (1) age ≥ 40 years; (2) a COPD diagnosis supported by pulmonary function tests of airflow obstruction even with a bronchodilator (forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) < 70%) when clinically stable for at least 3 months; (3) a primary diagnosis of AECOPD, defined as a deterioration of respiratory symptoms, such as dyspnea sensation, coughing, or purulent sputum that is beyond normal variability and severe enough to result in hospitalization [11]; and (4) meeting the diagnostic criteria for PH according to the 2015 European Society of Cardiology and the European Respiratory Society (ESC/ERS) Guidelines for the Diagnosis and Treatment of Pulmonary Hypertension Pressure diagnostic criteria [7], both of whom consider the diagnostic criteria for PH by echocardiography as follows: mild PH—36 mmHg $\leq$ PASP $\leq$ 50 mmHg; moderate PH—51 mmHg $\leq$ PASP $\leq$ 70 mmHg; and severe PH—PASP > 70 mmHg.

The exclusion criteria includes the following: (1) pregnant and lactating women; (2) idiopathic pulmonary hypertension; (3) other causes of pulmonary arterial hypertension (PAH), such as interstitial lung disease, congenital heart disease, heart valve disease, and acute left heart dysfunction; (4) suffering from other systemic diseases, such as left heart disease, autoimmune disease, blood system disease, thromboembolism disease, malignancy, and acute infectious diseases; and (5) patients who recently received a blood transfusion.

Our study protocol was approved by the ethics committee of Jiangsu Province Huaian No. 1 People's Hospital and was in agreement with the guidelines of the Declaration of Helsinki. An informed consent was not signed by each patient because of the retrospective design of this study.

2.2. Data Collection. The following clinical pathological data were obtained by reviewing the patients' medical records: age, gender, body mass index (BMI), smoking index, hospital stay duration, the course of the disease, underlying disease, and laboratory results during the first 12 hours after admission to the hospital. BMI is defined as a person's weight in kilograms divided by the square of the height in meters (kg/m^2). The definition of the smoking index is the average root number per day multiplied by years of smoking.

Inflammatory indices were calculated as follows: NLR = neutrophil counts/lymphocyte counts, PLR = platelet counts/lymphocyte counts, and SII = platelet counts $\times$ neutrophil counts/lymphocyte counts.

2.3. Statistical Analysis. All statistical analyses were performed using the Statistical Analysis System version 9.4 (SAS Institute, Cary, NC, USA). The Shapiro-Wilk method was used to test the normality of the data. Normally distributed numerical variables were presented as mean $\pm$ standard deviation, and the parameters which showed a nonnormal distribution were presented as median-interquartile range. Categorical variables were presented as frequencies and percentages. Normally distributed numerical variables were compared using the unpaired Student t-test. A Wilcoxon signed-rank test was used for the comparison of nonnormally distributed numerical variables which did not show a normal distribution after logarithmic transformation. Comparison of more than two independent groups was performed using the ANOVA and the Kruskal-Wallis test according to the distribution state. Differences between categorical variables were analyzed using a Pearson chi-square test. The correlation coefficients and significance of the continuous variables were assessed using a Spearman correlation test. Independent risk factors were analyzed by univariate and multivariate logistic regression. The Youden index method with a receiver operating characteristic (ROC) curve analysis was used to determine the optimal cut-off values of the predictive parameters of PH. The predictive probabilities were compared using the corresponding areas under the curve (AUCs) with 95% confidence intervals (CI). A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Subjects at Baseline. We retrospectively enrolled a total of 185 patients (age: 71.18 $\pm$ 8.17) with a diagnosis of AECOPD who met the inclusion criteria, including 141 males and 44 females (male proportion: 76.22%). 101 patients with PH secondary to COPD were included in the study group. PH was mild in 50 (49.50%) patients, moderate in 33 (32.67%), and severe in 18 (17.82%) patients in the study group. Baseline demographic characteristics and clinical data of the subjects reviewed are summarized in Table 1. The mean age and gender did not differ significantly between the study group and the control one (age: 72.06 $\pm$ 7.90 versus 70.12 $\pm$ 8.41, $p = 0.108$; male proportion: 76.24% versus 76.19%, $p = 0.994$).

Table 1: Baseline characteristics and clinical data of the enrolled subjects.

Characteristics	AECOPD group ($n = 84$)	PH group ($n = 101$)	p value
Age (years)	70.12 ± 8.41	72.06 ± 7.90	0.108
Gender (male), (n, %)	64 (76.19)	77 (76.24)	0.994
Hospital stay (day)	9.00 (7.00-11.00)	9.00 (7.00-10.00)	0.720
Course of disease (year)	10.00 (10.00-20.00)	10.00 (10.00-20.00)	0.537
BMI (kg/m^2)	23.68 ± 3.64	22.73 ± 3.99	0.095
Smoking index (year root)	600 (200-800)	600 (200-1000)	0.322
Hypertension (n, %)	35 (41.67)	36 (35.64)	0.402
Diabetes (n, %)	11 (13.10)	11 (10.89)	0.645
NYHA classification (n, %)			
I	29 (34.52)	7 (6.93)	
II	45 (53.57)	37 (36.63)	
III	10 (11.91)	48 (47.53)	
IV	0	9 (8.91)	

Abbreviations: AECOPD—acute exacerbation of chronic obstructive pulmonary disease; PH—pulmonary hypertension; BMI—body mass index; NYHA—New York Heart Association.

Confounding factors were compared, including the smoking index, BMI, hospital stays, and underlying disease. We did not find any differences in terms of BMI and smoking index between the two groups (all $p > 0.05$). Length of hospital stay, course of the disease, and coexisting illnesses (hypertension or diabetes) were not significantly different in patients with an exacerbation of COPD compared with those with PH. There was no difference in the demographic characteristics between the two groups, nor did they differ in confounding factors and comorbidities ($p > 0.05$). Therefore, the laboratory parameters were comparable.

3.2. Overall Comparison of the Laboratory Parameters and Baseline Echocardiographic Variables between the Study Group and the Control Group. The lymphocyte count was significantly decreased in the study group compared to the control one (0.91 versus 1.24, $p \leq 0.001$), but no significant differences among white blood cells, red blood cells, hemoglobin, neutrophils, platelets, and monocytes were presented between the two groups ($p > 0.05$) (Table 2).

As for the inflammatory indexes, patients with PH had a significantly higher median NLR value (6.52 versus 4.08, $p \leq 0.001$), higher median PLR value (220.88 versus 156.71, $p \leq 0.001$), and higher median SII value (1453.38 versus 884.87, $p \leq 0.001$) than the AECOPD group. Among the biochemical parameters, the NT-proBNP and albumin levels in the study group were significantly higher compared to those in the control one (653.00 versus 133.00, $p \leq 0.001$; 36.51 ± 4.75 versus 38.44 ± 3.78, $p = 0.003$). Furthermore, we found that the $PaCO_2$ value in the AECOPD group complicated by PH was higher compared with that in the AECOPD controls, 50.10 and 44.35, respectively ($p = 0.002$). Compared with the AECOPD group, the HCO_3^- value of the PH one was higher, 31.50 and 28.60, respectively ($p = 0.002$). The Lac of the study group was significantly higher than that of patients with COPD exacerbation (1.60 versus 1.50, $p = 0.032$).

Comparison of the D-Dimer levels of the two groups revealed that this value (0.65 versus 0.39, $p \leq 0.001$) was increased in the PH group compared to the AECOPD one. However, fibrinogen was similar in both groups (4.26 versus 4.30, $p = 0.708$). The estimated hemodynamic parameters by Doppler echocardiography of the two groups were also listed in Table 2. The right atrium diameter (RAD) and right ventricular diameter (RVD) were significantly higher in the study group compared with those in the control one (34.38 ± 6.60 versus 30.74 ± 3.80, $p \leq 0.001$; 18 versus 17, $p = 0.020$). The left atrium diameter (LAD), left ventricular end diastolic diameter (LVDD), and left ventricular ejection fraction (LVEF) of the two groups were not significantly different ($p > 0.05$).

To evaluate the association between inflammatory indexes and PH, we further compared the levels of NLR, PLR, and SII in patients categorized by PH severity. Patients with severe PH had a higher PLR than those with mild and moderate PH. PLR and p values for mild and moderate groups in comparison with the severe PH group (326.59) were as follows: mild PH, 210.64 ($p = 0.013$) and moderate PH, 210.31 ($p = 0.021$). As for NLR and SII, no significant differences were observed between either the mild or the moderate PH groups and the severe group. The Doppler echocardiography parameters of the PH group are listed in Table 3. LAD, LVDD, and LVEF of the three groups were not significantly different. PTRV and PASP were significantly higher in the severe group compared with the moderate and mild ones (4.31 versus 3.47 versus 2.90, $p \leq 0.001$; 79.50 ± 5.34 versus 58.18 ± 5.41 versus 42.98 ± 3.94, $p \leq 0.001$).

3.3. Association of the Comparable Data with the Estimated PASP and the NT-proBNP. The relationship between the estimated PASP (or NT-proBNP) and the laboratory parameters is shown in Table 4.

The laboratory parameters with differences between the two groups were further included in the correlation analysis with the estimated PASP and the NT-proBNP, including

Table 2: Comparison of the laboratory parameters and echocardiographic variables between the two groups.

Parameters	AECOPD group ($n = 84$)	PH group ($n = 101$)	p value
WBC ($\times 10^9$/l)	7.72 (5.83-9.99)	7.90 (6.79-10.41)	0.432
RBC ($\times 10^{12}$/l)	4.58 ± 0.59	4.55 ± 0.68	0.751
Hemoglobin (g/l)	136.21 ± 16.90	134.75 ± 19.14	0.586
Neutrophils ($\times 10^9$/l)	5.75 (4.18-7.89)	6.26 (4.85-8.17)	0.063
Lymphocytes ($\times 10^9$/l)	1.24 (0.95-1.59)	0.91 (0.66-1.26)	$p \leq 0.001$
Monocytes ($\times 10^9$/l)	0.52 (0.38-0.68)	0.54 (0.41-0.72)	0.576
Platelets ($\times 10^9$/l)	201.50 (165.00-252.50)	193.00 (154.00-229.00)	0.202
NLR	4.08 (2.89-7.26)	6.52 (4.95-12.28)	$p \leq 0.001$
PLR	156.71 (123.50-227.21)	220.88 (161.08-290.91)	$p \leq 0.001$
SII	884.87 (554.77-1453.34)	1453.38 (952.45-2441.84)	$p \leq 0.001$
Albumin (g/l)	38.44 ± 3.78	36.51 ± 4.75	0.003
NT-proBNP (pg/ml)	133.00 (76.00-238.50)	653.00 (167.00-1565.00)	$p \leq 0.001$
PH	7.41 ± 0.04	7.40 ± 0.05	0.080
$PaCO_2$ (mmHg)	44.35 (40.90-50.00)	50.10 (42.30-61.90)	0.002
HCO_3^- (mmol/l)	28.60 (26.90-31.20)	31.50 (27.30-37.40)	0.002
Lac (mmol/l)	1.50 (1.00-1.80)	1.60 (1.20-2.10)	0.032
D-Dimer (μg/ml)	0.39 (0.28-0.60)	0.65 (0.37-1.38)	$p \leq 0.001$
Fibrinogen (g/l)	4.30 (3.49-5.32)	4.26 (3.32-6.17)	0.708
LAD (mm)	27 (26-29)	29 (24-31.5)	0.217
LVDD (mm)	44.23 ± 4.41	43.40 ± 5.46	0.254
RAD (mm)	30.74 ± 3.80	34.38 ± 6.60	$p \leq 0.001$
RVD (mm)	17 (16-18)	18 (17-20)	0.020
LVEF	68 (66-68)	68 (65-68)	0.296

Abbreviations: AECOPD—acute exacerbation of chronic obstructive pulmonary disease; PH—pulmonary hypertension; WBC—white blood cell; RBC—red blood cell; NLR—neutrophil-to-lymphocyte ratio; PLR—platelet-to-lymphocyte ratio; SII—systemic-immune-inflammation index; $PaCO_2$—partial pressure of carbon dioxide; HCO_3^-—bicarbonate ion; Lac—lactic acid; LAD—left atrium diameter; LVDD—left ventricular end diastolic diameter; RAD—right atrium diameter; RVD—right ventricular diameter; LVEF—left ventricular ejection fraction.

lymphocytes, NLR, PLR, SII, NT-proBNP, $PaCO_2$, HCO_3^-, Lac, and D-Dimer. According to the Spearman correlation analysis, the estimated PASP was associated with NT-proBNP ($r = 0.500$, $p < 0.001$). There was a significant but weak correlation of PASP with lymphocytes ($r = -0.265$, $p = 0.007$), PLR ($r = 0.235$, $p = 0.018$), $PaCO_2$ ($r = 0.403$, $p < 0.001$), HCO_3^- ($r = 0.427$, $p < 0.001$), and D-Dimer ($r = 0.220$, $p = 0.027$), while there was no significant correlation with NLR, SII, and Lac. NT-proBNP showed a negative correlation with lymphocytes ($r = -0.386$, $p < 0.001$), and a positive correlation with NLR ($r = 0.340$, $p < 0.001$), PLR ($r = 0.355$, $p < 0.001$), SII ($r = 0.288$, $p < 0.001$), $PaCO_2$ ($r = 0.268$, $p < 0.001$), HCO_3^- ($r = 0.280$, $p < 0.001$), and D-Dimer ($r = 0.318$, $p < 0.001$).

3.4. Univariate and Multivariate Analysis of the Occurrence of Pulmonary Hypertension. The variables that were significantly different between the two groups were also tested in the univariate analysis. This analysis revealed that the factors impacting PH were lymphocytes, NLR, PLR, SII, NT-proBNP, $PaCO_2$, HCO_3^-, Lac, and D-Dimer (Table 5). The parameters identified as potential risk markers in the univariate analysis were further included in the multivariate logistic regression model ($p < 0.05$). Multivariate analyses identified NT-proBNP (OR: 1.003; 95% confidence interval (CI): 1.001-1.005; $p < 0.001$) as the independent risk factor correlated with PH. Nevertheless, NLR, PLR, and SII did not remain as independent predictors of PH.

3.5. Comparative Analysis of the Discriminative Ability of the Inflammatory Markers and NT-proBNP. A receiver operating characteristic curve (ROC) was generated to predict PH in AECOPD patients. The predictive accuracy values of the inflammatory markers and NT-proBNP are listed in Table 6.

Of the novel inflammatory markers, the NLR AUC (0.701; 95% confidence interval (CI), 0.629–0.766) was greater than that of PLR (AUC, 0.669; 95% CI, 0.596–0.736) and SII (AUC, 0.670; 95% CI, 0.597–0.737). The optimal cut-off value of NLR for predicting PH was 4.659, which yielded a 81.2% sensitivity and a 59.5% specificity. An SII of 1012 was considered the optimal cut-off value and the sensitivity and specificity were 70.3% and 59.5%, respectively. Using a PLR cut-off value of 160.0, the sensitivity and specificity for PH were 77.2% and 53.6%, respectively. The optimal cut-off value for NT-proBNP was 384.0 with a 58.4% sensitivity and a 92.9% specificity (AUC = 0.776). In order to

Table 3: Laboratory parameters and echocardiographic variables based on severity of PH.

	Mild PH ($n = 50$)	Moderate PH ($n = 33$)	Severe PH ($n = 18$)	p value
Lym ($\times 10^9$/l)	1.04 ± 0.43^{b}	1.00 ± 0.47^{c}	$0.68 \pm 0.37^{b,c}$	0.009
NLR	6.07 (4.86-11.25)	6.29 (5.05-10.62)	7.73 (4.80-17.73)	0.372
PLR	210.64 (153.61-277.05)^{b}	210.31 (160.63-263.37)^{c}	326.59 (232.77-443.02)^{b,c}	0.010
SII	1473.25 (813.27-2448.08)	1299.09 (932.72-2352.89)	1611.04 (1047.50-2999.36)	0.432
Albumin (g/l)	37.85 (34.65-41)	36.10 (32.60-39.35)	35.40 (33.43-36.85)	0.158
NT-proBNP (pg/ml)	237.50 (108-1050.25)^{a,b}	887 (274-3296)^{a}	1588 (587-5296)^{b}	$p \leq 0.001$
$PaCO_2$ (mmHg)	45.70 (39.25,51.38)^{a}	60.10 (49.55-72.05)^{a}	56.55 (40.85-63.78)	$p \leq 0.001$
HCO_3^- (mmol/l)	28.30 (26.70-32.20)^{a,b}	36.30 (32-40.60)^{a}	35.55 (28.13-39.43)^{b}	$p \leq 0.001$
Lac (mmol/l)	1.71 ± 0.57	1.58 ± 0.63	1.84 ± 0.90	0.389
D-Dimer (μg/ml)	0.52 (0.37-0.94)	0.93 (0.39-2.30)	1.15 (0.38-1.73)	0.099
LAD (mm)	27.34 ± 5.14	29.09 ± 5.37	30.06 ± 5.18	0.114
LVDD (mm)	43 (40-47)	45 (41-47)	42 (35-46.25)	0.190
RAD (mm)	$31.42 \pm 5.28^{a,b}$	36.33 ± 5.53^{a}	39 ± 7.90^{b}	$p \leq 0.001$
RVD (mm)	17 (16-18)^{a,b}	19 (17-22)^{a}	20.5 (17-32.5)^{b}	$p \leq 0.001$
PTRV (m/s)	2.9 (2.81-3.06)^{a,b}	3.47 (3.33-3.64)^{a,c}	4.31 (4.06-4.88)^{b,c}	$p \leq 0.001$
PASP (mmHg)	$42.98 \pm 3.94^{a,b}$	$58.18 \pm 5.41^{a,c}$	$79.50 \pm 5.34^{b,c}$	$p \leq 0.001$
LVEF	68 (65-68)	67 (65-68)	66 (65-68)	0.254

Abbreviations: Lym—lymphocytes; NLR—neutrophil-to-lymphocyte ratio; PLR—platelet-to-lymphocyte ratio; SII—systemic-immune-inflammation index; $PaCO_2$—partial pressure of carbon dioxide; HCO_3^-—bicarbonate ion; Lac—lactic acid; LAD—left atrium diameter; LVDD—left ventricular end diastolic diameter; RAD—right atrium diameter; RVD—right ventricular diameter; PTRV—peak tricuspid regurgitation velocity; PASP—pulmonary artery systolic pressure; LVEF—left ventricular ejection fraction. $^{a}p < 0.05$ for mild PH vs. moderate PH; $^{b}p < 0.05$ for mild PH vs. severe PH; $^{c}p < 0.05$ for moderate PH vs. severe PH.

Table 4: Relationship between the statistically different indicators and NT-proBNP (or PASP).

Parameters	NT-proBNP		PASP	
	r value	p value	r value	p value
Lymphocyte (10^9/l)	-0.386	<0.001	-0.265	0.007
NLR	0.340	<0.001	0.087	0.389
PLR	0.355	<0.001	0.235	0.018
SII	0.288	<0.001	0.069	0.494
NT-proBNP (pg/ml)	1	—	0.500	<0.001
$PaCO_2$ (mmHg)	0.268	<0.001	0.403	<0.001
HCO_3^- (mmol/l)	0.280	<0.001	0.427	<0.001
Lac (mmol/l)	0.122	0.100	0.013	0.894
D-Dimer (μg/ml)	0.318	<0.001	0.220	0.027

Abbreviations: PASP—pulmonary arterial systolic pressure; $PaCO_2$—partial pressure of carbon dioxide; HCO_3^-—bicarbonate ion; NLR—neutrophil-to-lymphocyte ratio; PLR—platelet-to-lymphocyte ratio; SII—systemic-immune-inflammation index; $PaCO_2$—partial pressure of carbon dioxide; HCO_3^-—bicarbonate ion; Lac—lactic acid.

improve the diagnostic efficacy of COPD-related pulmonary hypertension, we further examined the feasibility of the combined prediction of NLR and NT-proBNP. The prediction accuracy of NLR combined with NT-proBNP (AUC = 0.813) was higher than that of NLR or NT-proBNP alone. Figure 1 shows the ROC curves of the predictive parameters of PH in patients with AECOPD.

4. Discussion

This study showed that NLR, PLR, and SII were significantly higher in PH patients secondary to COPD than in the AECOPD controls. In addition, these markers can be used to predict PH in AECOPD patients. In these cases, NLR has been shown to be superior to PLR and SII in its discriminative ability.

PH induced by COPD can lead to increased pulmonary arterial pressure, elevated pulmonary vascular resistance, and progressive right heart failure, which results from increasing right ventricular afterload. The progress of PH is associated with a significant increase in clinical deterioration and risk of death. The pathogenesis of PH is due to the maladaptation of various vasomotor factors secreted by injured endothelial cells, resulting in early pulmonary vasoconstriction and later pulmonary vascular remodeling. Increasing evidence suggests that inflammation plays an extremely decisive role in the progression of PH [12]. The pathophysiology of pulmonary vascular remodeling in PH is not only the pathological damage of endothelial cell function but also the excessive perivascular infiltration of inflammatory cells [13].

Lymphocytes decline in autoimmune diseases and are responsible for peripheral immune tolerance. Consistent with previously published literature [14, 15], the current study showed that lymphocyte counts in PH patients were significantly lower compared with those in the control AECOPD group, which might be able to reflect the balance between host inflammatory status and immune status. The classification of T lymphocytes in PH patients is obviously different from that

Table 5: Univariate and multivariate analysis of the effects of the baseline parameters on PH.

Factors	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p value	OR (95% CI)	p value
Lymphocyte (10^9/l)	0.226 (0.114, 0.448)	<0.001	1.055 (0.273, 4.078)	0.938
NLR	1.173 (1.081, 1.273)	<0.001	1.161 (0.924, 1.458)	0.200
PLR	1.006 (1.003, 1.009)	<0.001	1.003 (0.993, 1.013)	0.564
SII	1.001 (1.000, 1.001)	<0.001	0.999 (0.998, 1.001)	0.256
NT-proBNP (pg/ml)	1.003 (1.001, 1.004)	<0.001	1.002 (1.001, 1.003)	<0.001
$PaCO_2$ (mmHg)	1.047 (1.019, 1.075)	<0.001	1.018 (0.939, 1.104)	0.664
HCO_3^- (mmol/l)	1.103 (1.042, 1.167)	<0.001	0.981 (0.822, 1.170)	0.828
Lac (mmol/l)	1.911 (1.130, 3.234)	0.016	1.663 (0.837, 3.305)	0.146
D-Dimer (μg/ml)	1.910 (1.235, 2.953)	0.0036	1.581 (0.960, 2.603)	0.072

Abbreviations: PH—pulmonary hypertension; $PaCO_2$—partial pressure of carbon dioxide; HCO_3^-—bicarbonate ion; NLR—neutrophil-to-lymphocyte ratio; PLR—platelet-to-lymphocyte ratio; SII—systemic-immune-inflammation index; Lac—lactic acid; CI—confidence intervals; OR—odds ratio.

Table 6: Comparison of the discriminative ability of NLR, PLR, SII, and NT-proBNP to predict PH.

Parameters	NLR	PLR	SII	NT-proBNP
Cut-off value	4.659	160.0	1012	384.0
AUC	0.701	0.669	0.670	0.776
95% CI	0.629, 0.766	0.596, 0.736	0.597, 0.737	0.709, 0.834
Sensitivity (%)	81.2	77.2	70.3	58.4
Specificity (%)	59.5	53.6	59.5	92.9
Positive predictive value (%)	70.7	66.7	67.6	90.8
Negative predictive value (%)	72.5	66.2	62.5	65.0
Accuracy (%)	71.4	66.5	65.4	74.1
Associated criterion	0.407	0.308	0.298	0.513
N	181	108	167	128

Abbreviations: NLR—neutrophil-to-lymphocyte ratio; PLR—platelet-to-lymphocyte ratio; SII—systemic-immune-inflammation index; AUC—area under the curve; CI—confidence interval.

of the healthy population. Studies on the lymphocyte subsets in patients with PH are controversial. Stacher et al. [16] discovered that in different types of pulmonary hypertension, almost all of them were accompanied by a large number of inflammatory cells (mainly lymphocytes) infiltrating into the lung perivascular region and the interstitium. Another study showed that $CD8^+$ cytotoxic T cells were reduced and regulatory T cells were increased in patients with idiopathic pulmonary hypertension [17]. Furthermore, researchers have found that the level of Th17 cells and interleukin-17A (IL-17A) increased in PH patients associated with connective tissue disease [18] and idiopathic pulmonary hypertension (IPH) [19], which suggested that Th17 cells may play a crucial role in promoting the development of PH. An upregulation of $CD25^{+-}$ $Foxp3^+$ cells in $CD8^+$ T cells and a downregulation of $CD4^+CD25^+Foxp3^+$ T cells were also observed in PAH patients compared to healthy controls by Zhu et al. [20].

There was no significant difference of blood neutrophil level between the non-PH group and the PH group in AECOPD patients in our study. However, neutrophil infiltration has been observed in murine lungs in hypoxia-induced PH mice [21], and the role of neutrophils in the pathogenesis of PH was not fully understood. A study demonstrated that circulating inflammatory mediators have been associated with poor clinical outcomes in PH [22]. Neutrophils release a consistent amount of reactive oxygen species (ROS) and further trigger massive amplification of the inflammatory cascade reaction by activating mitogen-activated protein kinase (MAPK) and redox-sensitive transcription factors [23]. IL-6, secreted by neutrophils, promotes pulmonary artery smooth muscle cell (PASMC) proliferation by upregulating the expression of vascular endothelial growth factor (VEGF) and downregulating the expression of pulmonary bone morphogenetic protein receptor type 2 (BMPR2) [24]. Soon et al. [25] observed that IL-6, IL-8, TNF-α, and other inflammatory factors were significantly higher during the development of PH than in the normal population. There are several reasons that can explain our results. Firstly, the sample size was small and may have affected the research result. Secondly, the treatment received with corticosteroids before admission may have affected the white blood cell counts [26]. Thirdly, the patients in this study were older and may have been less responsiveness to inflammation.

In this study, NLR, PLR, and SII were all significantly higher and the result was consistent with established associations between PH and host immune and inflammatory

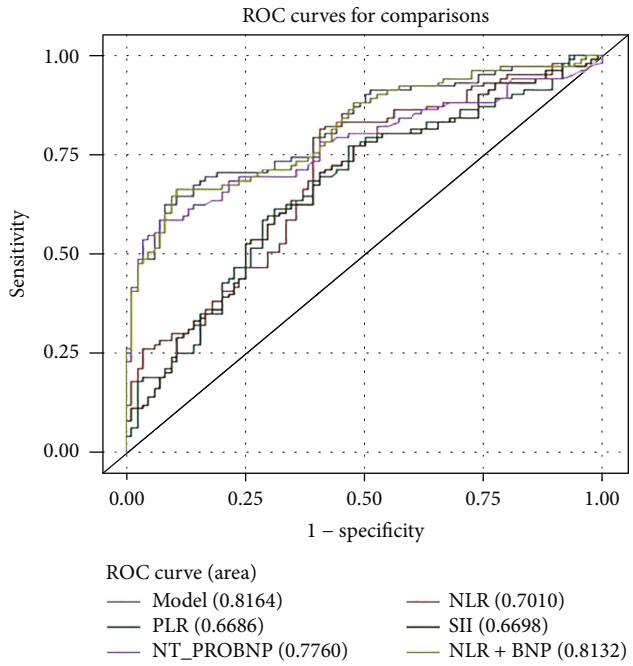

Figure 1: ROC curves for determining the cut-off value of NLR, PLR, SII, and NT-proBNP for predicting PH in AECOPD patients. Abbreviations: NLR—neutrophil-to-lymphocyte ratio; PLR—platelet-to-lymphocyte ratio; SII—systemic-immune-inflammation index.

environments. NLR, based on neutrophil count and lymphocyte count, has been increasingly investigated as a marker of systemic inflammation, especially because it is a relatively inexpensive and widely available evaluation tool. Recently, NLR has been extensively studied in COPD. Several studies have shown that NLR was linked with disease severity and may be useful in the prediction of the prognosis of COPD. Gunay et al. [27] found that compared with stable COPD patients (NLR = 2.59), the NLR value of the AECOPD group was significantly increased (NLR = 4.28), and the NLR value of COPD patients was significantly higher than that of the healthy control group (NLR = 1.71). Yao et al. [28] discovered that higher levels of NLR (>6.24) and PLR (>182.68) predicted an increased risk of hospital mortality in the patients with AECOPD. For the first time, a study demonstrated a significant increase in NLR values in patients with PAH compared with healthy volunteers [14]. Özpelit et al. subsequently reported that NLR may be directly related to the severity and prognosis of PAH [15]. Nevertheless, few studies have concentrated on the predictive ability of NLR in PH patients induced by COPD. In this study, the level of NLR was significantly higher in PH patients compared with AECOPD patients. The ROC curve analysis showed that the AUC of the NLR for predicting PH was greater than that of PLR and SII, and the predictive ability of the NT-proBNP was stronger than NLR. However, for some community hospitals with backward medical facilities, NLR is easy to calculate from a routine complete blood count without increasing the patients' burden and is considerably cheaper than NT-proBNP. Use of NLR for predicting PH resulted in a greater sensitivity than for NT-proBNP (81.2% versus 58.4%), but NT-proBNP had a higher associated specificity of 92.9% in this cohort. The combination of NLR and NT-proBNP resulted in an AUC of 0.813. Thus, we can infer that NLR may be a more objective indicator of the balance between host inflammatory and immune responses than indicators such as PLR or SII.

To our knowledge, PLR and SII have not been studied in PH patients induced by COPD until now. We discovered that PLR and SII increased significantly in patients complicated with PH than in the AECOPD group. COPD patients have a hypercoagulable state due to long-term bed rest, hemodynamic abnormalities, and the hypoxia of cells. The platelet-related index can effectively evaluate the severity of COPD. PLR, based on platelet and lymphocyte count, was increased in AECOPD patients than in COPD and healthy controls and has been proven to be linked with poor prognosis in COPD patients [29]. The systemic-immune-inflammation index (SII), based on lymphocyte count, neutrophil count, and platelet count, is a comprehensive indicator with an important prognostic value for colorectal cancer [30], resectable pancreatic cancer [31], gastric cancer [32], and so on. Few studies have been concerned with the association between the novel inflammation-based biomarkers and the severity of PH in AECOPD combined with PH patients. We further evaluated the relationship between these biomarkers and the estimated PASP. As a result, these markers have no significant correlation with estimated PASP other than PLR, but were significantly correlated with NT-proBNP, a well-known factor that can predict disease progression in PH patients. From this, we can conclude that NLR and SII can be used for the early prediction of patients with PH, but have no statistically significant correlation with the severity of PH.

Blood gas parameters were also compared. Owing to some patients needing oxygen intake or invasive mechanical ventilation for a long time after admission, the partial pressure of arterial oxygen in the blood gas analysis was disturbed. Therefore, PH, $PaCO_2$, and HCO_3^- were utilized in our study. The $PaCO_2$, HCO_3^-, and Lac values of the PH group were higher than those of the control one. Spearman's correlation analysis showed that the estimated PASP was positively correlated with $PaCO_2$ and HCO_3^-. These results suggested that $PaCO_2$ and HCO_3^- may be related to the severity of pulmonary artery pressure, in addition to NLR or SII. In accordance with this, Samareh conducted a cross-sectional study of 1078 patients with severe PH in COPD [33]. This study illustrated that various factors, such as hypoxia and hypopnea, play a major role in the severity of PH in these patients. Under the influence of hypoxemia and hypercapnia, pulmonary vascular resistance is significantly increased due to pulmonary vasoconstriction or even vasospasm. As the disease progresses, pulmonary vascular remodeling eventually leads to PH.

5. Strengths and Limitations of This Study

There are some strengths and limitations to our study. First, this article maybe one of the few researches investigating NLR, PLR, and SII as novel inflammation-based biomarkers in patients with PH secondary to COPD. These markers can be regarded as a promising and convenient tool to predict PH in COPD patients. Second, some studies indicated

that NLR is influenced by age and BMI [34, 35]. Therefore, in the clinical use of these indicators, it is still necessary to comprehensively consider the patient's age, medical history, BMI, etc. Our matching process adequately controlled for the potential confounders to make these novel markers more reliable. The limitations are as follows: First, our study was a single-center one with a small sample size, which means that the study sample included patients who are cared for by a single tertiary medical center. In addition, considering the critical condition of part of the AECOPD patients, lung function tests were not performed for the sake of these patients' safety. Second, invasive examination would not be indicated and ethical for all admitted COPD patients, and the estimated PASP measured by Doppler echocardiography was only moderately correlated with the values conducted by right heart catheterization. Third, the symptoms and quality of life expressed as St. George's Respiratory Questionnaire (SGRQ), Modified British Medical Research Council (mMRC) Questionnaire, and COPD Assessment Test (CAT) scores and the history of previous deteriorations could not be obtained due to its retrospective design.

6. Conclusion and Future Directions

From this study, we concluded that NLR, PLR, and SII can be used as practical means for the prediction of PH especially in community hospitals with poor medical infrastructures and the accuracy of NLR was higher than that of PLR and SII. The threshold of NLR was 4.659 for the early differential screening between AECOPD patients complicated by PH and patients with AECOPD alone. Given the grave prognosis of PH, larger multicenter, well-designed, prospective clinical studies are warranted to validate the use of these promising biomarkers, which are routinely measured on admission and require no extra cost in clinical practices. Understanding the critical role of the inflammatory signaling pathway in the pathophysiological mechanisms of PH may also lead to potential therapeutic targets in the future.

Data Availability

The data used to support the findings of this study are included within the supplementary information file.

Conflicts of Interest

The authors report no conflict of interest.

Supplementary Materials

Patients diagnosed with AECOPD ($n = 185$) were registered in this retrospective study. All patients evaluated for PH in our study underwent Doppler echocardiography and were divided into study and control groups depending on whether they also had PH. 101 AECOPD patients with PH were included in the PAH group, and the remaining eighty-four patients were assigned to the COPD group. Clinical characteristics and baseline laboratory tests (routine blood test (RBT), blood gas analysis, and amino terminal pro-B-type natriuretic peptide (NT-proBNP)) were tested at enrollment. All these data were listed in the supplementary file. There are some things particularly revelatory here: (1) in the gender column, 1 is for male and 2 is for female; (2) in the Respiratory failure, Hypertension, and Diabetes columns, 1 is for no and 2 is for yes; (3) BMI is defined as a person's weight in kilograms divided by the square of the height in meters (kg/m^2); (4) the definition of the smoking index is the average root number per day multiplied by years of smoking; (5) inflammatory indices were calculated as follows: NLR = neutrophil counts/lymphocyte counts; PLR = platelet counts/lymphocyte counts; SII = platelet counts × neutrophil counts /lymphocyte counts; (6) abbreviations: LAD—left atrium diameter; LVDD—left ventricular end diastolic diameter; RAD—right atrium diameter; RVD—right ventricular diameter; PTRV—peak tricuspid regurgitation velocity; AECOPD—acute exacerbation of chronic obstructive pulmonary disease; PH—pulmonary hypertension; BMI—-body mass index; WBC—white blood cell; RBC—red blood cell; NLR—neutrophil-to-lymphocyte ratio; PLR—platelet-to-lymphocyte ratio; SII—systemic-immune-inflammation index; $PaCO_2$—partial pressure of carbon dioxide; HCO_3^-—-bicarbonate ion; Lac—lactic acid; PASP—pulmonary arterial systolic pressure; $PaCO_2$—partial pressure of carbon dioxide; HCO_3^-—bicarbonate ion; NLR—neutrophil-to-lymphocyte ratio; PLR—platelet-to-lymphocyte ratio; SII—systemic-immune-inflammation index. *(Supplementary Materials)*

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