# Clinical significance of measuring inflammatory markers in patients with pulmonary arterial hypertension

**To the Editor** Pulmonary arterial hypertension (PAH) is characterized by perivascular inflammatory infiltrates and elevated levels of certain cytokines.[1] These abnormalities are more advanced in patients with a mutation in the bone morphogenetic protein receptor type 2 (BMPR2), which significantly increases the risk of idiopathic PAH. The BMPR2 mutation has been reported in 11% to 40% of subjects with idiopathic PAH, while the dysfunction of the BMPR2 signaling pathway has been observed in all types of PAH.[2] As the expression of the receptor activator of nuclear factor-κB ligand (RANKL) is induced by BMPR2, a recent study by Jasiewicz et al.[3] who investigated the role of soluble RANKL (sRANKL) and its decoy receptor, osteoprotegerin (OPG), in patients with PAH is of particular importance.[3] They showed that the levels of sRANKL and OPG were higher in patients with PAH compared with controls and correlated with markers of disease severity. However, the practical value of their results is still unclear.

Theoretically, some markers of inflammation specific for PAH could serve as screening tests for this disease in patients with unexplained breathlessness or in high-risk groups such as patients with connective tissue disease, congenital heart disease, portal hypertension, HIV infection, and others. Experimental pulmonary hypertension suggests that altered immunity is a cause rather than consequence of vascular disease; therefore, early detection of PAH-specific inflammatory changes could potentially enable the causal treatment of the disease.

The clinical effect of the modulation of the immune system in patients with PAH by PAH-specific therapies (prostanoids, endothelin receptor antagonists, and phosphodiesterase inhibitors) is poorly understood. Although the findings from basic science studies are encouraging, clinical studies show unequivocal results.[4] Recently, we have shown that a 3-month treatment of patients with idiopathic PAH with specific therapies have not changed the plasma level of interleukin 6, the expression of which is also modulated by BMPR2.[5] The currently enrolling clinical trials in PAH with molecules such as FK506, rituximab, and anakinra selectively targeting the immune system are promising. In the study by Jasiewicz et al.,[3] 10 patients were incident cases who received PAH-targeted therapy at entry to the study. It would be interesting to see whether the levels of OPG and sRANKL changed in this group during the 6-month follow-up.

Finally, different subtypes of PAH are characterized by different mechanisms and inflammatory profiles. The development of PAH associated with congenital heart disease is triggered by mechanical overload of the pulmonary vessels resulting from an increased flow through the pulmonary circulation, while some molecular mechanisms are thought to be involved in the initiation and progression of idiopathic PAH. Jasiewicz et al.[3] did not report any significant differences in serum OPG and sRANKL concentrations between patients with idiopathic PAH, PAH associated with congenital heart disease, and PAH associated with connective tissue disease. Does it mean that their results can be generalized to the whole PAH population or maybe the numbers of study groups were too small to prove heterogeneity of OPG and RANKL levels in different PAH etiologies?

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**Authors' reply** We are grateful to Drs. Kopeć and Podolec for their insightful comments concerning our paper. We agree with the authors that there are still numerous questions about the usefulness of inflammatory biomarkers in the diagnosis of pulmonary arterial hypertension (PAH). Our study was designed to investigate potential new pathogenic mechanisms based on the clinical assessment of patients with PAH and not to provide novel biomarkers for clinical use. Therefore, clinicians should interpret our findings with caution. The promising results of the receiver-operating characteristic curve analysis of soluble receptor activator of nuclear factor-κB ligand (sRANKL) in the identification of individuals with PAH (C-statistics, 0.809; 95% confidence interval, 0.714–0.904; a cut-off value of 2.51 pmol/l for a sensitivity of 92% and specificity of 62%),[1] require validation in larger prospective and community-based studies. We emphasize that osteoprotegerin (OPG)–sRANKL signaling is altered in patients with PAH and both elements of this axis may provide different and complementary information about the pathogenesis and clinical course of the disease. Still this description should not be directly applied in current clinical practice.

The therapy of PAH may modulate the immune response by various mechanisms. It may be a result of hemodynamic stabilization,[2] unspecific for the type of therapy. There also may be an immunomodulatory effect of particular drugs such as prostacyclins.[4] Therefore, in order to study the effect of therapy on the activation of inflammatory markers, appropriate measures must be taken. In our study, we were not prepared to compare the effects of particular drugs on the changes in OPG and sRANKL concentrations. In general, in patients who were enrolled before the start of a specific PAH therapy, neither OPG nor sRANKL significantly changed after 6 months of follow-up (OPG, 3.94 ±1.22 pmol/l at baseline vs 3.61 ± 1.31 pmol/l, $P = 0.31$; sRANKL, 10.19 ±11.44 pmol/l at baseline vs 28.15 ±39.85 pmol/l, $P = 0.15$, respectively). In the case of sRANKL, the heterogeneity was so extreme that an apparently large increase was not statistically significant. Owing to a small number of incident cases, we were unable to analyze this phenomenon in detail; however, this may be due to a differential response to treatment,[3] different specific treatments applied (prostacyclin vs sildenafil vs bosentan),[3] or the effect of estrogens.[4] It will be particularly important to describe the effect of treatment on the mechanisms such as OPG-RANKL signaling that may contribute to the development of PAH.

There were no significant differences in either OPG or RANKL concentrations between the groups of patients with different etiologies of PAH. An average concentration of OPG in patients with connective tissue disease (CTD) was 3.63 ±1.31 pmol/l in comparison with 4.19 ±2.06 pmol/l in patients with idiopathic PAH and 3.98 ±2.37 pmol/l in patients with congenital heart disease. sRANKL was also similar in all 3 groups: in patients with CTD, it was 9.03 ±9.78 pmol/l; in idiopathic PAH, 6.1 ±4.16 pmol/l; and in patients with congenital heart disease, 7.26 ±5.52 pmol/l. Apparently, the higher values in patients with CTD are offset by more pronounced heterogeneity of sRANKL concentrations within this group.

We would like to stress that our study was not designed to assess subgroup effects but rather to generate new hypotheses to be analyzed in detail in animal models,[5] which might provide further insight into what we could expect in humans and later to be applied in clinical practice.


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