Vitamin D binding protein: a new biomarker of disease severity in lymphangioleiomyomatosis

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Vitamin D binding protein is a novel biomarker of disease severity in LAM http://ow.ly/qGdc30ml0EE

Cite this article as: Liu H-J, Henske EP. Vitamin D binding protein: a new biomarker of disease severity in lymphangioleiomyomatosis. *Eur Respir J* 2018; 52: 1801886 [https://doi.org/10.1183/13993003.01886-2018].

Lymphangioleiomyomatosis (LAM) is a rare destructive pulmonary neoplasm that occurs primarily in women, with onset typically during the child-bearing years. LAM is caused by hyperproliferation of abnormal smooth muscle-like "LAM cells" carrying inactivating mutations in the tumour suppressor genes encoding tuberous sclerosis complex protein 1 or 2 (TSC1 or TSC2) [1–4]. LAM cells are believed to destroy the surrounding lung parenchyma, leading to cystic lung destruction, pneumothorax and progressive lung function decline [5]. A pivotal clinical trial has demonstrated that rapamycin (sirolimus) can stabilise lung function in LAM [6]. It remains difficult to identify which women with early stage LAM are at highest risk of future lung function decline. Current predictors of lung function decline include premenopausal status and elevated vascular endothelial growth factor-D (VEGF-D) levels [7–9]. The identification of biomarkers of disease progression and/or disease activity could further enable personalised therapy for LAM, and could streamline clinical trial design by identifying cohorts of women with the highest likelihood of disease progression, thereby decreasing the duration, cost and number of patients needed [10].

In this issue of the *European Respiratory Journal*, Miller *et al.* [11] have taken advantage of carefully banked specimens from the National Centre for LAM in the UK as well as samples collected at the National Heart Lung and Blood Institute (NHLBI) in the USA. The authors used a mass spectrometry proteomics approach to survey proteins in the serum from LAM patients and controls, identifying vitamin D binding protein (VTDB) as a novel biomarker of disease severity and clinical outcome in LAM. 126 proteins were detected in all patients (n=50) and controls (n=20) in the initial discovery cohort. VTDB was the most significantly downregulated serum protein ($log_2FC=2.6$) in LAM patients compared to controls. Lower levels of VTDB were confirmed by ELISA.

The mechanisms underlying the decreased VTDB levels are not yet fully understood. The authors performed genotyping studies on all participants from the NHLBI cohort and 65 participants of European origin from UK cohort, and discovered that the allele frequencies of single nucleotide polymorphisms (SNPs) within three VTDB haplotypes were not different in the LAM patients compared to the general population, suggesting that genotype, at least at these alleles, is not responsible for the lower VTDB levels in LAM *versus* controls.

Received: Oct 04 2018 | Accepted after revision: Oct 21 2018

In the discovery cohort, lower levels of VTDB were associated with progressive disease (>50 mL per year loss of forced expiratory volume in 1 s (FEV_1)) at the time of enrolment. Importantly, the investigators had access to longitudinal clinical data from these patients, with 144 patient-years of observation for the UK cohort (mean 19 months per patient) and 500 patient-years for the NHLBI cohort (mean 40 months per patient). Serum VTDB was not associated with prospective changes in FEV_1 or time of death or lung transplantation. However, in the NHLBI cohort, a surprising association was found between VTDB genotype and median time of death or transplant, with a more rapid time to death/transplant in women with the CC genotype at rs4588 *versus* AA or AC (104 months *versus* 150 months). This exonic SNP has been previously associated with lower serum vitamin D levels compared with CA or AA genotype at rs4588 [12], suggesting increased death/transplant time may be associated with decreased bioavailability due to lower binding affinity of VTDB to vitamin D.

The fact that serum VTDB levels were not associated with age, age at diagnosis, menopausal status, nature of presenting symptoms, the presence of tuberous sclerosis, angiomyolipomas, lymphatic disease or serum VEGF-D level suggest it may present as a unique marker of disease progression.

From a mechanistic perspective, how could serum VTDB levels and/or genotype(s) be associated with the progression of LAM? We know that LAM cells express oestrogen receptor-α (ER-α) and progesterone receptors [13], and preclinical studies utilising mouse models have demonstrated that oestrogen enhances the survival and metastasis of TSC2-deficient cells [14]. Interestingly, in other oestrogen-responsive disease, such as breast cancer, vitamin D has anti-proliferative activity by inducing cell cycle arrest *via* downregulation of ER-α expression [15]. It is possible that high serum VTDB allows more vitamin D to be available to the LAM cells, thereby slowing their proliferation.

Matrix metalloproteinase (MMP)-2 and MMP-9 are expressed by LAM cells [16] and are known to degrade elastin and collagens, thus contributing to connective tissue and alveolar remodelling in LAM. In TSC2-deficient models, MMP-2 and MMP-9 are overexpressed in an oestrogen-dependent manner [17, 18]. Although serum levels of MMP-2 and MMP-9 do not correlate with lung functions, MMP-9 expression in LAM biopsy specimens correlates with pulmonary function parameters, including FEV_1 and diffusing capacity of the lung for carbon monoxide [19, 20]. Vitamin D can inhibit the conversion of metalloproteinases including MMP-2 and MMP-9 to their active forms in lung fibroblasts [21]. Thus, a high serum VTDB could contribute to increased accessibility of vitamin D within the lung, thereby suppressing matrix metalloproteinase activity and lessening tissue destruction.

In addition to being a carrier for vitamin D, VTDB has anti-inflammatory and immunoregulatory functions [22] which may directly impact the immune microenvironment in LAM. This is of particular interest, because recent studies have begun to elucidate the immune microenvironment of LAM [23–25] alongside work in mouse models supporting the potential of checkpoint blockade immunotherapy treatment in LAM [26, 27]. Interestingly, two VTDB haplotypes (*GC1F* and *GC1S*) are linked to enhanced macrophage activation in comparison with the *GC2* haplotype [28]. VTDB can also act as a chemotactic factor by binding to complement component 5a (C5a), leading to increased recruitment of neutrophils and macrophages [29, 30]. These studies suggest that VTDB may offer protection against LAM progression *via* effects on the innate immune system.

In summary, Miller *et al.* [11] have elegantly identified serum VTDB as a new candidate biomarker of LAM associated with disease progression, and in parallel identified a SNP in the VTDB haplotype as a genetic biomarker of time to transplant/death. At this point, it is not clear whether these two findings (the serum levels and the genotype) are linked. An important caveat is that serum VTDB levels were not associated with all prospective changes in lung function in either the NHLBI or the UK cohort. If validated in other cohorts, one might envision a time when these and other biomarkers of disease activity and progression (including VEGF-D, menopausal status and other factors) [19] could be combined into a "LAM Progression Score", with critical implications for personalised therapeutic decision-making and clinical trial design.

Conflict of interest: None declared

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