Serum sCD163 as a biomarker of adipose tissue inflammation in obstructive sleep apnoea patients: limits and perspectives

To the Editor:

Recently, MURPHY *et al.* [1] demonstrated, through a challenging animal, cellular and human translational approach, that intermittent hypoxia induces a pro-inflammatory activation of adipose tissue macrophages (ATMs), promoting insulin resistance. They confirmed that intermittent hypoxia lowers insulin-mediated glucose uptake in 3T3-L1 adipocytes, and evidenced the interrelationship between inflammation and insulin resistance in the adipose tissue of mice exposed to intermittent hypoxia. They then measured the concentration of serum soluble CD163 (sCD163), an assumed pro-inflammatory biomarker reflecting macrophage activation, in obstructive sleep apnoea (OSA) patients classified according to their BMI and apnoea/hypopnoea index (AHI). This is the first time serum sCD163 has been evaluated in OSA patients, and its association with AHI is promising. With the aim of enhancing its relevance in further studies, we wish to discuss the following points.

1) Activation of adipose tissue macrophages (ATMs) towards M1 phenotype in OSA patients. MURPHY *et al.* [1] made the assumption that circulating sCD163 levels in OSA patients reflect the intermittent hypoxia-dependent polarisation of ATMs towards a M1 phenotype. As appropriately cited, KRAČMEROVÁ *et al.* [2] showed that circulating sCD163 concentrations are associated with both macrophage content in human adipose tissue, and insulin sensitivity. However, the assumption that serum sCD163 reflects such a polarisation based on the ADAM-17-dependent cleavage, as hypothesised by ETZERODT *et al.* [3], is unconvincing since only shown in HEK293 cells. Human ATMs are characterised by a high expression of CD163 which, in contrast, is weakly expressed in differentiated M1 macrophages [4]. Therefore, the question arises as to whether a link exists between the ATM/M1 difference in CD163 expression and the increase in serum sCD163 concentration and, if confirmed, to what extent nocturnal intermittent hypoxia impacts this inflammatory activation. An interesting, but technically demanding, approach would be to reproduce, both on control and OSA patients, the experiments from ZEYDA *et al.* [4] on isolated subcutaneous ATMs, using flow cytometry for ATM/M1/M2 characterisation (including CD163) and quantifying *in vitro* their cytokine production under stimulating agents, in addition to sCD163 measurements both in serum and in culture supernatants. Besides the ATM/M1 ratio in CD163 expression, ZEYDA *et al.* [4] showed that ATMs produce higher levels of tumour necrosis factor-α, interleukin (IL)-6 and macrophage inflammatory protein-1α under interferon-γ stimulation, and IL-10 under lipopolysaccharide (LPS) stimulation, compared to M1 macrophages. In contrast, under LPS stimulation, M1 macrophages produced high amounts of Rantes, a chemokine not secreted by ATMs. Accordingly, it could be assumed that high circulating Rantes levels observed in obesity hypoventilation syndrome mainly result from an ATM to M1 phenotypic change [5]. Thus, although promising, the association between serum sCD163 and AHI requires further investigation before suggesting sCD163 as a circulating biomarker of ATM activation in OSA patients.

2) Relationship between ATM-specific inflammation and insulin-resistance in OSA patients. MURPHY *et al.* [1] supported the hypothesis of an intermittent hypoxia-dependent ATM activation through a multiple regression with sCD163 as a dependent variable, from which AHI (or oxygen desaturation index) emerged as the only predictor. Unfortunately, it seems that the authors did not include the homeostasis model assessment resistance index (HOMA-IR) as an independent variable in this model, making it difficult to appreciate if sCD163-based inflammation is related to insulin resistance in these patients. Moreover,

results from univariate regressions between serum sCD163 and body mass index (BMI), waist/hip ratio, HOMA-IR or lipid parameters are lacking, making it difficult to evaluate the relationship between sCD163 and insulin resistance. Indeed, such data could confirm the recent results from CINKAJZLOVÁ et al. [6], who showed plasma sCD163 as positively associated with BMI, waist/hip ratio, blood glucose, insulin, and HOMA-IR, and negatively associated with high density lipoprotein-cholesterol in a group of subjects including controls, obese and diabetic patients. Besides these statistics, the authors could also provide serum sCD163 mean±SD for the BMI- and AHI-based subgroups, which would allow us to compare the ranges from healthy subjects, (334±25 ng·mL^{-1}), obese (467±40 ng·mL^{-1}) and diabetic patients (514±37 ng·mL^{-1}) [6], or from many others pathophysiological contexts [7]. Further studies could include potential correlations of serum sCD163 with more sophisticated anthropometric measurements, such as fat mass.

3) Potential impact of the liver on the relationship between serum sCD163 and OSA severity. Finally, MURPHY et al. [1] relevantly reminded that, besides adipose tissue disorders, intermittent hypoxia also unbalances hepatic metabolism, favouring steatohepatitis and insulin resistance [8]. Accordingly, an increase in liver enzymes has been shown in OSA [9, 10], but still remains controversial. Recently, alanine aminotransferase has been found as positively associated with plasma sCD163 in obese subjects [6]. Thus, although patients with OSA were monitored for liver function, it would have been interesting to present liver enzyme results, and to test their associations with the circulating levels of sCD163.

In conclusion, MURPHY et al. [1] highlighted for the first time the potential interest of serum sCD163 as a pro-inflammatory biomarker associated with OSA severity. However, further investigations are needed to confirm the assumption that it reflects the impact of intermittent hypoxia on ATM polarisation towards the M1 phenotype.

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From the author:

I thank D. Monneret and J-P. Bastard for their interest in our manuscript [1] and I could not agree more with their comment that the role of soluble CD163 (sCD163) in obstructive sleep apnoea (OSA) requires further detailed evaluation. As outlined in our article, the measurement of sCD163 in our patient cohort

followed our results from the *in vitro* and *in vivo* studies demonstrating M1 polarisation of adipose tissue macrophages in response to intermittent hypoxia and thus, was not an *a priori* hypothesis of our study. Nonetheless, the detected independent association of sCD163 with OSA severity is intriguing.

CD163 is a haemoglobin scavenger receptor that is expressed by cells of the monocyte-M2-macrophage lineage [2]. The soluble form of CD163 is present in plasma of healthy individuals [3] but in response to several inflammatory mediators, levels rise acutely due to metalloproteinase-17/tumour necrosis factor-α-converting enzyme (ADAM17/TACE)-mediated cleavage [4–6]. sCD163 has emerged as a sensitive biomarker of inflammation and increased levels have been found in numerous pro-inflammatory conditions, *i.e.* sepsis, hepatitis, rheumatoid arthritis, scleroderma or atherosclerosis [7, 8]. Moreover, sCD163 is increasingly linked with adipose tissue inflammation and is considered a prognostic predictor for the development of type 2 diabetes [9–11]. Taken all this information together, sCD163 is a very attractive target in OSA.

In our cross-sectional study, univariate analysis revealed a significant correlation of sCD163 with all OSA severity parameters but also with body mass index and waist/hip ratio. However, in linear regression, adjusted for anthropometric and demographic parameters, the apnoea/hypopnoea index (AHI) (or oxygen desaturation index, when substituted for AHI) remained the only significant predictor of this response. Hence, these results along with the pre-clinical data generate the hypothesis that sCD163 may serve as a potential pro-inflammatory marker and predictor of metabolic consequences in OSA. However, testing this hypothesis, as acknowledged in our manuscript, warrants further targeted evaluation including large prospective studies.

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