Pseudoneutropenia in lymphangioleiomyomatosis (LAM) patients receiving sirolimus: evaluation in a 100 patient cohort

To the Editor:

Diurnal variation in white blood cells (WBC), particularly neutrophils, is well-described [1]. WBC levels are lower in the morning and increase through the day [1, 2]. Drugs with immunosuppressive effects, such as sirolimus, may further lower WBC counts. This phenomenon has been observed in clozapine and related atypical antipsychotic medications, drugs with known immunosuppressive effects [3, 4]. For patients receiving these drugs, blood counts measured in the early morning may lead to a false impression of low WBC/neutrophil counts ("pseudoleukopenia/pseudoneutropenia") [4–8] that may result in discontinuation or a reduction in dose and suboptimal treatment. Of importance, isolated morning neutropenia is not known to increase the risk of infection [6, 9].

Lymphangioleiomyomatosis (LAM) is a rare, multisystem disease, characterised by cystic lung destruction, lymphatic infiltration and renal angiomyolipomas. It may occur sporadically or in association with tuberous sclerosis complex, an autosomal dominant, neurocutaneous disorder [10, 11]. The disease is treated with inhibitors of mechanistic target of rapamycin (mTOR), such as sirolimus (rapamycin) or everolimus [10–14]. Sirolimus is an immunosuppressive agent that inhibits activation and proliferation of T-cells and B-cells by reducing interleukin-2 production, and has been approved by the Food and Drug Administration for use in transplant recipients for over 17 years [15, 16]. Sirolimus has been recently approved for use in LAM [17]. In a prior study evaluating the sustained effects of sirolimus in LAM, neutropenia/leukopenia was reported in 40% of patients, and upper respiratory tract infections were reported in 66% [12]. To avoid increased risk of infection while on the drug, systemic immunity is monitored in part by obtaining a complete blood count (CBC) with differential. Diurnal variation may not be appreciated in the interpretation of low WBC counts.

We report here a patient with LAM (female, age 53 years) receiving sirolimus who presented in the morning (06:00 h) with low WBC and neutrophil counts (3.18×10^3 μL^{-1} and 1.54×10^3 μL^{-1}, respectively). Previously, neutropenia caused the primary physician to decrease the dose of sirolimus. Repeat of the cell counts later in the day showed an increase in WBC and neutrophil counts by 42% (4.53×10^3 μL^{-1}) and 88% (2.91×10^3 μL^{-1}), respectively. These WBC and neutrophil levels would not warrant modification of the sirolimus dose.

The objective of this study was to determine if LAM patients experience pseudoleukopenia and/or pseudoneutropenia, particularly when they are on sirolimus treatment, and whether this phenomenon is associated with increased incidence or severity of infection. To test this hypothesis, we examined a cohort of 100 LAM patients either treated or not treated with sirolimus. All patients participated in NHLBI Protocol 95-H-0186, and gave written informed consent before enrolment. We compared leukocyte counts at three time-points throughout the day. Since diurnal effects are also affected by food intake, particularly lipids, we measured leukocyte counts prior to meals and following breakfast and lunch [18]. In patients
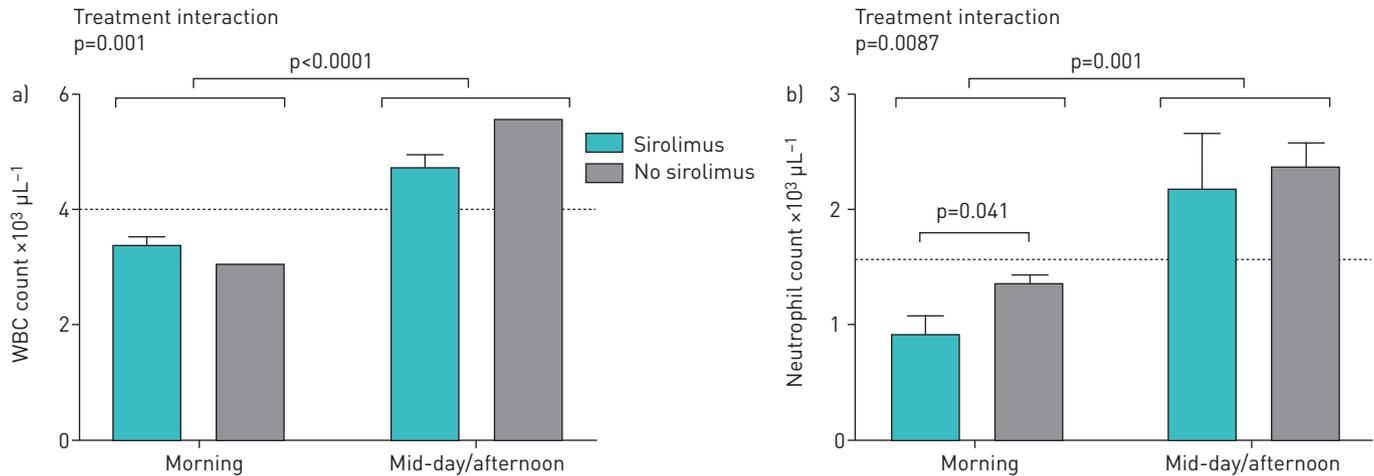
FIGURE 1 a) White blood cell (WBC) counts for 10 lymphangioleiomyomatosis (LAM) patients (nine on sirolimus, one off sirolimus) experiencing pseudoneutropenia in the morning and during mid-day or afternoon. The dotted line is the lower threshold of the normal range for WBC counts $(3.98 \times 10^3 \mu L^{-1})$. b) Neutrophil counts for eight LAM patients (four on sirolimus, four off sirolimus) experiencing pseudoneutropenia in the morning and during mid-day or afternoon. The dotted line is the lower threshold of the normal range for neutrophil counts $(1.56 \times 10^3 \mu L^{-1})$. Comparisons between morning and mid-day/afternoon values were conducted with an unpaired t-test and assessment of treatment interaction was conducted with two-way repeated measures ANOVA.

that experienced pseudoleukopenia and/or pseudoneutropenia, incidence and severity of infection was collected up to 1 year prior to the study date and up to 1 year after the study date.

100 female patients with LAM (mean age 50.8±10.4 years) were provided a breakfast and lunch of their choosing after a fasting period of ∼7 h. Blood samples were taken at three times: morning (06:00 h ±1.5 h) prior to breakfast, mid-day (11:00 h ±1.5 h) about 2 h after breakfast, and afternoon (15:00 h ±1.5 h) about 1 h after lunch. A CBC with differential and lipid panel was obtained at each measurement. ANOVA was used to analyse results within and between patients and, in addition, based on sirolimus use (55 patients receiving sirolimus, 45 patients not receiving sirolimus). The National Institutes of Health Clinical Center indicates the normal range of WBC counts and neutrophil counts as $3.98–10.04 \times 10^3 \mu L^{-1}$ and $1.56–6.13 \times 10^3 \mu L^{-1}$, respectively. As such, pseudoleukopenia was defined as a change in WBC count from $<3.98 \times 10^3 \mu L^{-1}$ in the morning to $>3.98 \times 10^3 \mu L^{-1}$ at mid-day or in the afternoon. Likewise, pseudoneutropenia was defined as a change in neutrophil count from $<1.56 \times 10^3 \mu L^{-1}$ in the morning to $>1.56 \times 10^3 \mu L^{-1}$ at mid-day or in the afternoon.

Among patients receiving sirolimus, 16.4% (nine out of 55 patients) presented with pseudoleukopenia, while 2.2% of patients not receiving sirolimus (one out of 45 patients) presented with pseudoleukopenia. 7.3% of patients receiving sirolimus (four out of 55 patients) presented with pseudoneutropenia, while 8.89% of patients not recieiving sirolimus (four out of 45 patients) presented with pseudoneutropenia. However, neutrophil counts were 35% lower in the morning (p=0.041) in patients on sirolimus experiencing pseudoneutropenia compared to patients off treatment experiencing pseudoneutropenia (figure 1b). Notably, multivariate analysis showed that treatment status significantly affected variation in WBC and neutrophil count between the morning and mid-day/afternoon (p⩽0.0087) (figure 1).

Among the 14 patients who experienced pseudoneutropenia/pseudoleukopenia, incidence of infection was evaluated 8.9±3.0 months prior to the study date (n=11 patients) and 7.7±2.4 months after the study date (n=10 patients). The severity of all reported infections was mild based on the Common Terminology Criteria for Adverse Events (supplementary material) [19]. Rates of infection between patients receiving sirolimus and not treated with sirolimus were similar (25% in patients off sirolimus and 20% in patients on sirolimus) (supplementary material).

In the larger patient cohort (n=100 patients), absolute counts for WBC, neutrophils and monocytes were lowest in the morning, and increased later in the day (p<0.0001) (figure 2). Eosinophil counts decreased from the morning to the early afternoon (p<0.0001). Significant changes in monocyte count throughout the day were only observed in patients on sirolimus (p<0.0001). Lymphocyte, basophil and immature granulocyte counts did not show significant variation throughout the study period. Triglyceride levels increased throughout the day; however, total cholesterol levels were not statistically different.

Our results show that some LAM patients on sirolimus experience pseudoleukopenia and pseudoneutropenia. Given the transient nature of this event and the importance of sirolimus therapy in
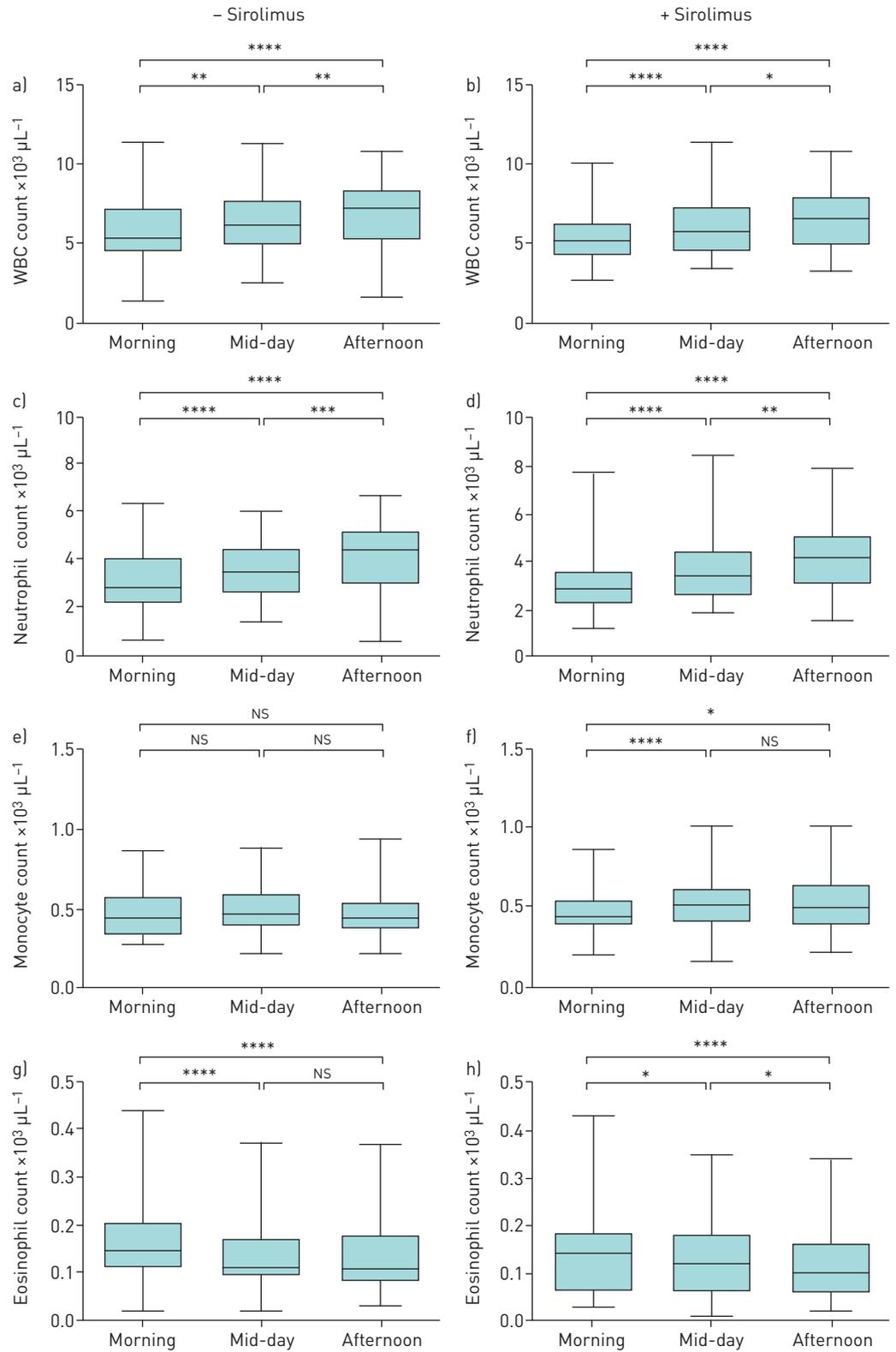
FIGURE 2 Comparison of absolute counts for a, b) white blood cells (WBC), c, d) neutrophils, e, f) monocytes, and g, h) eosinophils for 100 patients with lymphangioleiomyomatosis (LAM) (44 not receiving sirolimus treatment (a, c, e and g), 55 receiving sirolimus treatment (b, d, f and h)) at three time-points during the day. Box-and-whisker plots show median values, upper and lower quartiles, and highest and lowest values. ANOVA was used to analyse results within and between patients. *: $p \leqslant 0.05$; **: $p \leqslant 0.01$; ***: $p \leqslant 0.001$; ****: $p \leqslant 0.0001$; NS: $p > 0.05$.

stabilising lung function, distinguishing between pseudoleukopenia and persistent sirolimus-induced leukopenia is critical. In patients experiencing pseudoleukopenia or pseudoneutropenia, infection rates were low and similar in patients both on and off treatment. Furthermore, all infections reported about 9 months prior to the study date and 8 months after the study date were mild. As such, we confirm that isolated morning neutropenia or leukopenia did not increase risk of infection. If morning WBC or neutrophil counts are less than the normal range, we suggest repeating CBCs later in the day and using those results as a better metric of sirolimus toxicity.

Diurnal variation in WBC counts is well documented, and is linked with cyclic changes in adrenocorticotropic hormone and cortisol levels, which peak in the morning [1]. This hormonal variation probably promotes redistribution of leukocytes within a 24-h period. While this variation was generally observed in all patients, our results further show that sirolimus treatment may enhance cyclic changes in WBC and neutrophil count. This is indicated by significant treatment interactions in WBC and neutrophil counts between morning and mid-day/afternoon values in patients experiencing pseudoleukopenia or pseudneutropenia (figure 1). These results suggest an effect of sirolimus on circadian pathways. There is known involvement of mTOR signalling in the suprachiasmatic nuclei (SCN) of the hypothalamus, the source of the mammalian circadian clock [20]. mTOR is a downstream target of the p42/44 mitogen-activated protein kinase (MAPK) signalling pathway, and light triggers coordinate cyclic AMP-response element binding protein (CREB) and mTOR activation in SCN neurons [20]. The data suggest that inducible translation, mediated by a light-responsive mTOR cascade in the SCN, contributes to the circadian clock entrainment process. Thus, modification of this mTOR signalling through sustained sirolimus treatment could modify diurinal variation in WBC counts.

The observed decrease in eosinophil count from morning to early afternoon supports previous studies exploring the role of the circadian clock in eosinophils and mast cells, and could explain why allergic diseases are frequently exacerbated when eosinophil counts peak between midnight and early morning [21].

In conclusion, although WBC counts must be monitored when treating patients with immunosuppressive drugs, transient leukopenia in the morning may be a result of circadian rhythm, and leukocyte counts may recover later in the day. This may not necessarily warrant a decrease in drug dose. Diurnal variation should be considered when morning WBC counts are used as the basis for decreasing treatment dose. Currently, morning pseudoneutropenia has only been reported with antipsychotic medications; however, our findings show that this phenomenon is present with sirolimus as well [5, 9].

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