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Auto-and Crosscorrelation Analysis to Assess Diurnal Plasma Cortisol Levels and Routine Differential Blood Count Parameters

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Summary. **The diurnal courses of cortisol and differential blood count were determined at 08.00 H, 12.00 H, 16.00 H and 20.00 H in 14 healthy male subjects on two subsequent days. Data were analysed descriptively (mean/SD, linear regression) and sine wave models were fitted. Periodicity was investigated by means of autocorrelation. Relations between cortisol levels and hematology parameters were assessed using a crosscorrelation technique. Neutrophils correlated significantly with cortisol levels with a time lag of less than 4 hours. In contrast, the correlation of lymphocytes and cortisol was characterized by a time lag of 4 hours and that of eosinophils at a lag time of 4 hours. In conclusion, hematology parameters and plasma cortisol appear to be closely related within a complex feedback network. The character of this interaction between hormonal and hematology parameters may have clinical relevance, and they may replace cortisol measurements if the correlations can be well established.**

Key words—cortisol, differential blood count, autocorrelation, crosscorrelation, healthy volunteers.

INTRODUCTION

Diurnal variations of cortisol levels and differential blood count (DBC) results are well-known chronobiological phenomena[1,2]. Maximum cortisol levels are found at the beginning of the activity period. Patients with depression show hypercortisolism, although this knowledge has not yet been implemented in routine diagnostic assessment in psychiatry. The peak values of the leucocyte totals appear in late evening hours. The maximum values (acrophase) of neutrophils occur in the afternoon (approx. at 15.00 H). The maximum values for lymphocytes are found in the resting phase around midnight and 01.00 H. Recent findings indicate that subsets of lymphocytes show different circadian rhytms[3]. While subtypes such as CD8+ of gammadelta T cells show an increase in the daytime, other subtypes such as CD4+ or B cells show their maximum during night. Eosinophil counts generally reach a maximum during the resting phase, although eosinophil counts are characterized by marked variability.

The present pilot study in healthy volunteers aims to assess the relation between diurnal cortisol and parameters of the differential blood count using crosscorrelation techniques as a basis for further chronobiological studies in order to assess the synchronization of patients e.g. with psychosis by means of routine hematology parameters.

METHODS

Diurnal changes in plasma cortisol and differential blood count (DBC) parameters (total leucocytes [White blood count, WBC], neutrophils, lymphocytes) were analysed every 4 hours (08.00-20.00 H) in 14 healthy male volunteers (age: 21-35 years) over 2 days. Cortisol was determined with commercial RIA kits (intra- and interassay variation less than 10%) and DBC using a hematology analyser (Technicon H1 Hematology Analyser). Data were analysed descriptively (Mean, SD, CV [coefficient of variation= SD/mean*100%]) using Statistica (Statsoft Inc.,

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Tulsa, OK, USA). Linear or non-linear regressions for the circadian time (t) course of an effect Y(t) were calculated as follows: $Y(t)=a+b*\sin((2\pi*t/d)+c)$. The parameter a characterizes the MESOR (Midline Estimatating Statistic of Rhythm). As the time intervals were not equidistant, the MESOR cannot be interpreted as the mean value over 24 hours. The parameter b denotes the amplitude of the sine curve, d denotes the length of one period, and c the phase of the model with regard to the reference time. Corresponding p-values (t test) for each parameter of the model were given for assessment of goodness of fit, with and $p<0.05$ (*) considered as significant (Table Curve2D, SPSS Inc., Chicago, USA).

Autocorrelation functions (ACF) as well as crosscorrelation functions (CCF) including approximated standard errors for plasma cortisol and DBC values were calculated. A detailed description of time series analysis is given in Chatfield[4] and Kendall[5]. Autocorrelation analysis, i. e. correlation of a signal with itself when the signal is shifted with time lags, is particularly useful to detect periodicities of signals and to estimate the proportion of the noise of a signal. The function is symmetrical with regard to time $t=0$, where it has its maximum. If the ACF dies out, it is a clear hint that the process is stationary and that the series is defined by its mean, variance, and ACF. In cybernetic systems the speed of dying out gives information about the time constant of the mechanism or the "noise", which in extreme cases, results in a mere Dirac function. The ACF can be defined as the correlation of order k (lag) between each t^{th} element of the series. One lag in our study corresponds to 4 hours (lags 1,2 and 3; 5, 6 and 7 and so on) or 8 hours (lags 4, 8 and so on) due to the different intervals between blood samples. The basic equation for calculation of the autcorrelation function ACF_k for descrete data is given by the following formula, where m is the mean of yt:

$$ACFk=\sum_{t=k+1}^{n}(yt-m)*(yt-k-m)/\sum_{t=1}^{n}(yt-m)^2$$

In the case of crosscorrelograms, the correlation between two signals is assessed as a function of the corresponding time lag k (CCFxy), where mx and my correspond to the respective means of the series. CCF need not be symmetric with regard to lag=0. It also allows detection of the rhythms of the basic signals. The general function is defined as follows:

$$CCFxy=\sum_{t=1}^{n-1}(xt-mx)*(yt+k-my)/n$$

As the time intervals between blood samples were 4 hours, the crosscorrelation can detect time lags of 4/-4 hours and above.

RESULTS

As expected, the maximum mean concentration of plasma cortisol was found at 08.00 H. Total leucocytes, neutrophils, lymphocytes and eosinophils showed peak values of the mean at 20.00 H, 12.00 H, 20.00 H and 08.00 H, respectively. Fig.1 presents the diurnal variation of the neutrophils and total white blood count (leucocytes). No significant sine wave model could be calculated for diurnal variations of either leucocytes or neutrophils via non-linear regression. In Fig. 2a and b, sine wave models for lymphocytes and eosinophils are depicted, which underlined the circadian periodicity of these differential blood count parameters.

The overall coefficient of variation for diurnal cortisol levels was 51.8%. There was also marked interindividual variation of differential blood count characteristics, the overall coefficient of variation for diurnal controls being 20.5% (WBC), 26.4% (neutrophils), 23.8% (lymphocytes) and 37.7% (eosinophils). Despite this marked interindividual variation of both differential blood count characteristics and plasma cortisol, linear regression showed correlations between cortisol levels and total leucocytes ($r=0.14$) or neutrophils ($r=0.23$*), whereas lymphocytes and and eosinophils did not show a linear correlation with cortisol levels, which corresponds well with CCF values at time lag 0.

Evident periodic patterns in the ACF were found for cortisol levels and lymphocytes (Fig. 3 a, b and c), whereas neutrophils and eosinophils did not reveal such a rhythmic course. The ACF of cortisol showed significant maximum values after 4 time lags, i. e. after 24 hours. In contrast, with regard to lymphocyte (0.545), eosinophil (0.554) and neutrophil (0.698), count maximum autocorrelation values were found for 1 time lag, i. e. these parameters of the differential blood count generally resemble those of the previous observation. The maximum of the ACF of the total white blood count (leucocytes) was 0.685, which falls between the values of the leucocyte subsets.

The maximum value of the CCF for neutrophils (0.2285) versus cortisol was found at a lag time of zero and showed continuous positive values with comparable high values for lags of -1 and $+1$, i. e. after shifts of $+4$ and -4 hours (Fig. 4a). The maximum of the CCF for lymphocytes (0.197) was observed 4 hours (one time lag) later and was negative at lag intervals of 12 hours (Fig. 4b). For eosino-

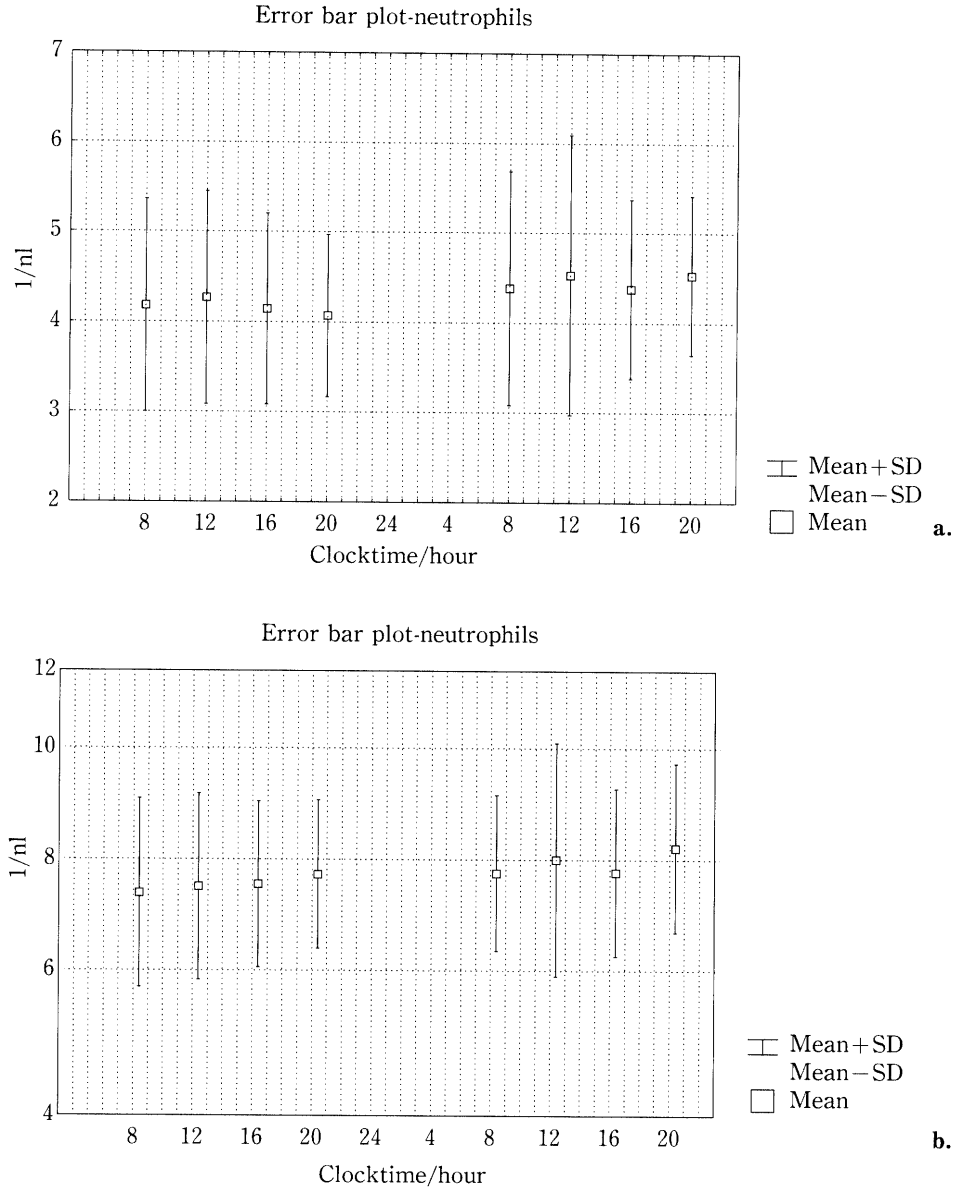
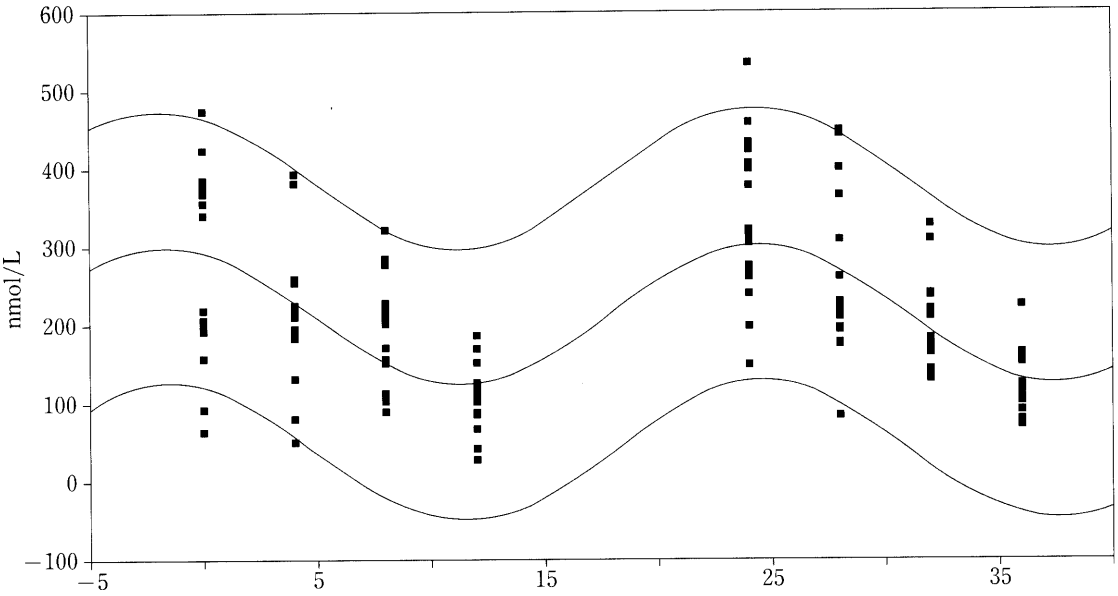


Fig. 1. Diurnal course (mean, SD) of neutrophil and leucocyte count in healthy male volunteers over two days. Although a circadian rhyhthm is probable, no significant sine wave model could be fitted to the data (p-values > 0.05).

phils the maximum CCF value (-0.309) was found at a lag of -4 hours ($-$one time lag), being positive at lag intervals of 8 to 12 hours (Fig. 4c).

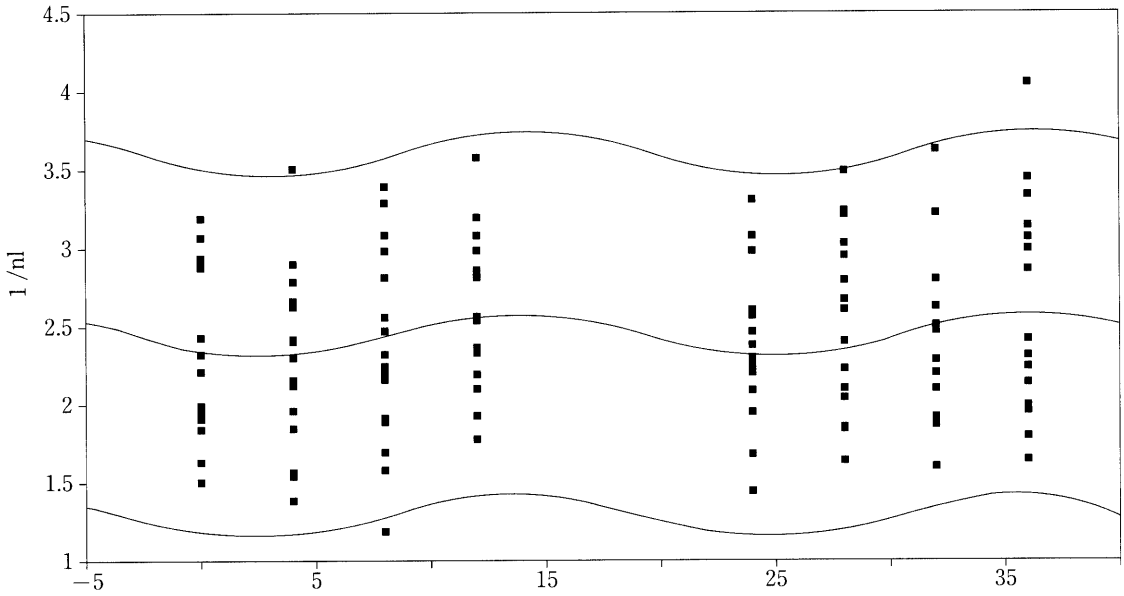
DISCUSSION

The time series analysis confirms the diurnal pattern of differential blood count parameters in healthy subjects. Differences of acrophases, particularly the early peak value of neutrophils, may be due to lack of synchronisation of the volunteers, although this does not interfere with the objective of the study, which sought to show time-specific dependencies between cortisol on the one hand and differential blood count parameters on the other. The ACF showed a clear rhythmic pattern of cortisol and verified that values at 8.00 H correlated best after a time shift of 24 hours. In contrast, lymphocytes correlate best with the observation recorded just 1 time lag (i. e. 4 hours)



a.

Cortisol levels over a period of two days
sine wave model
0.00 Ref-Time=8.00 clocktime day 1-24.00 Ref-Time=8.00 clocktime day 2
a=212.1 nmol/L b=88.5 nmol/L
C=1.98 h d=26.07 h



b.

Lymphocyte count over a period of two days
sine wave model
0.00 Ref-Time=8.00 clocktime day 1-24.00 Ref-Time=8.00 clocktime day 2
a=2.45 1/nl b=0.14 1/nl
C=3.901 h d=22.16 h

Fig. 2. Legends on the following page.

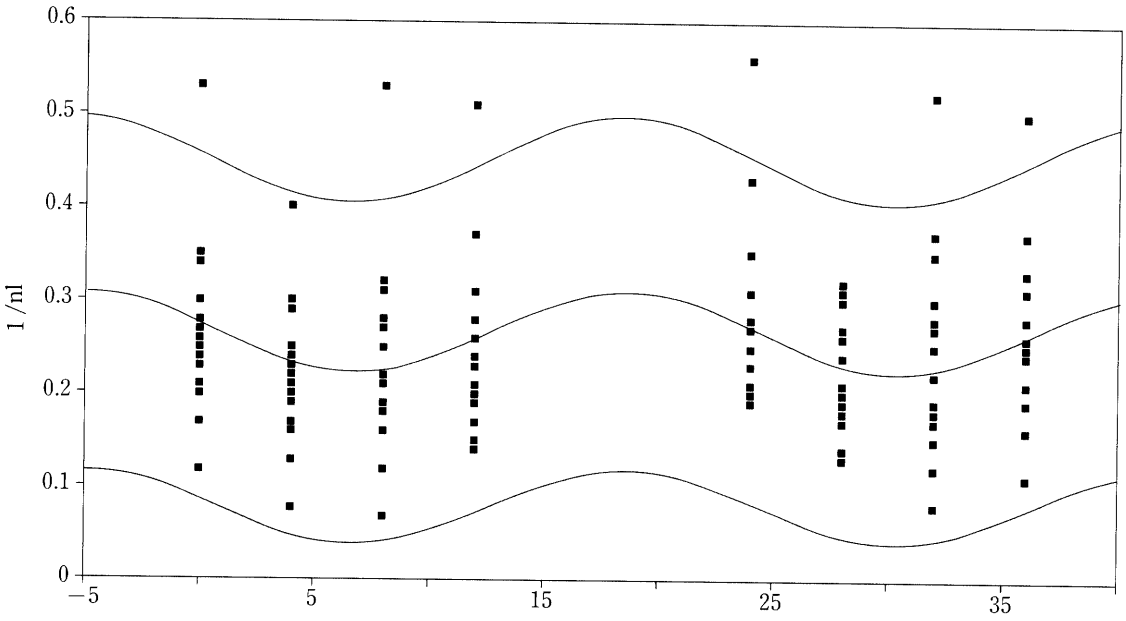


Fig. 2. Course of plasma cortisol and differential blood count parameters (lymphocytes and eosinophils) in healthy male volunteers over two days presented as chronograms including 95%- prediction intervals. Significant circadian sine wave models (p-values < 0.05) could be fitted to the data. The periods d lasted approximately between 22 to 26 hours.

before. This result was also found for total leucocytes and eosinophils. One explanation for this observation is that cortisol is governed by a strong autonomous oscillator and is independent from other pacemakers, whereas lymphocyte count, and possibly other differential blood count parameters, are under the control of various factors including cortisol levels.

Although the time intervals were not identical, significant sine wave models could be fitted. Due to the lack of observations during the night, the MESOR cannot be interpreted as the mean value over one period. However the models were able to predict peak values (acrophases) and trough values (bathyphases) quite reliably. The peak values of lymphocytes during early evening and night hours were predicted by the model as well as was the acrophase of eosinophils during night. With regard to cortisol levels, sine wave models appear to be in adequate to predict the course of the concentration over a period of 24 hours. Particularly, the low values at midnight and the following hours were not well predicted, so that other models such as the sum of exponentials may be more suitable to describe the circadian course of cortisol levels. However, the sine wave model was able to assess correctly the acrophase during the early morning hours. The lack of fit for the diurnal course of neutrophils clearly demonstrated the limits of the sine wave model. This result corresponds with the well-known leucocytosis inducing effect of corticosteroids which is used therapeutically. Interestingly, this effect was detected by means of CCF, although no significant sine wave model of neutrophils could be calculated.

Cortisol rises during and after physical activity and lasts several days until cortisol levels return to the initial value[6]. Cortisol levels are modified by endocrinological diseases or during therapy with steroid, and play an increasing role in the diagnostics of psychoses. The diurnal cortisol secretion is furthermore influenced by other conditions such as bright light[7], serotoninergic drugs[8], breast cancer[9] and age[10]. After physical activity the white blood count, and particularly leucocytes, show higher counts, which may not return to normal within 24 hours[6].

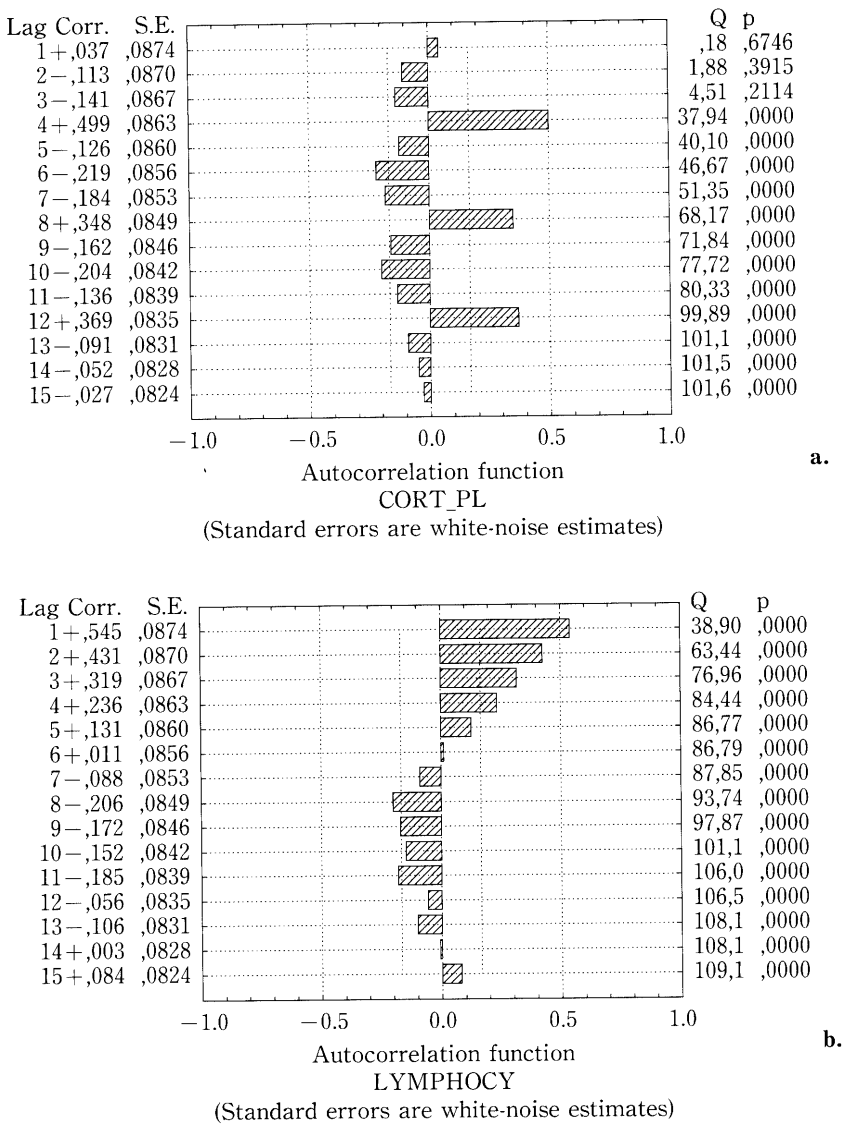
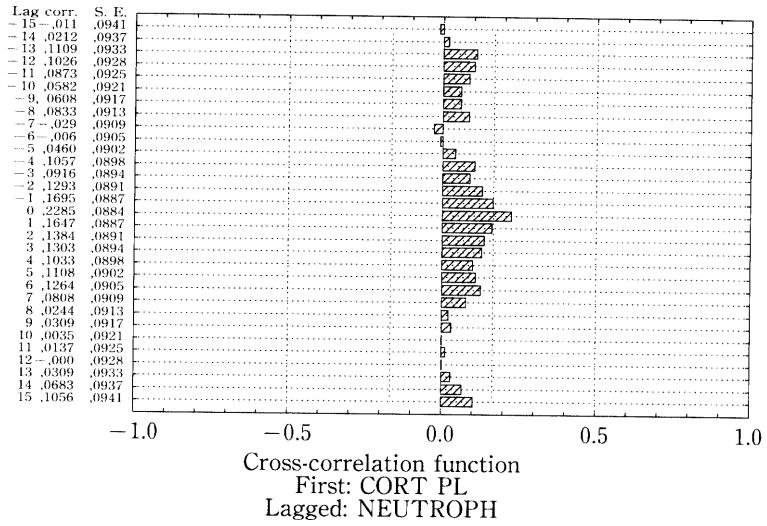


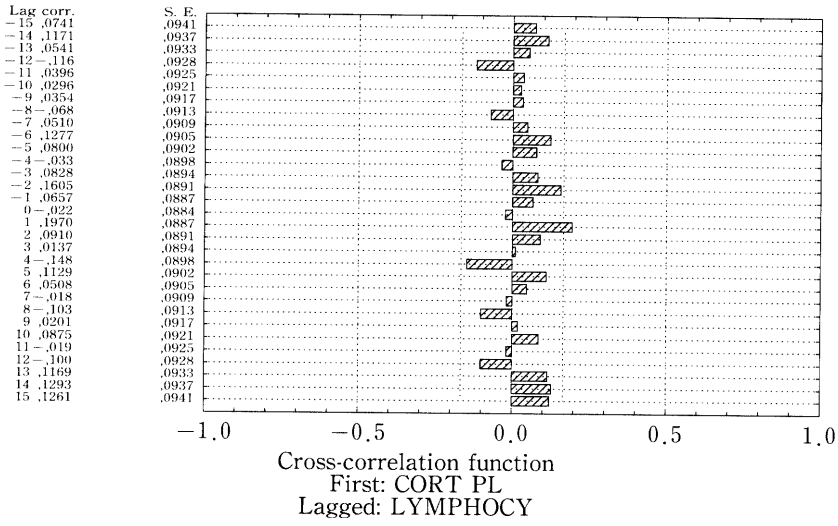
Fig. 3. Autocorrelation functions (ACF) of cortisol and lymphocytes based on diurnal data from 08.00 H to 20.00 H. Both ACFs indicate an evident periodic diurnal pattern of the basic data. Though cortisol levels correlate best at time lags of 24 hours, lymphocytes tend to correlate well with the immediately preceding observation.

Obviously, differential blood count parameters also show clinically important diurnal variation, which is clearly related to plasma cortisol levels. Therefore, time points of collecting blood samples have an essential influence on the result and interpretation we obtain. The analysis of both cortisol and differential blood count may give additional information on the desynchronisation of an individuum, which can be induced by factors such as diseases, physical stress, or jet-lag.

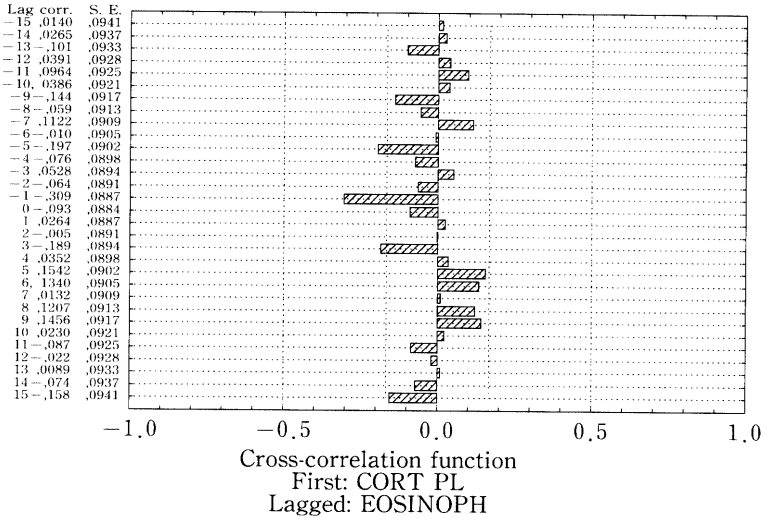
The results of CCF analysis show cortisol correlated with differential parameters either without a time lag of 4 hours or above (neutrophils) or with a time lag of at least 4 hours (lymphocytes, eosoinophils). Obviously, linear regression methods were not able to dectect correlations, if time shift is an influencing factor. Both autocorrelation and crosscorrelation can be calculated even if the time intervals between blood samples are not identical, on the assumption that the different values of time lags are taken into considera-



a.



b.



c.

Fig. 4. Crosscorrelation functions (CCF) of cortisol plasma levels versus neutrophil, lymphoyte, and eosinophil count. Neutrophils appear to be closely related to plasma cortisol, i.e. a maximum correlation was found for a time lag of 0, although no significant sine wave model could be fitted to the data (see Fig. 1).

tion. However, this study also shows that final investigations should be done also during night hours and at narrower time intervals or an order of 2 hours. Time intervals of 2 hours would also improve the power of the auto- and crosscorrelation analysis to characterize periodicities and relations.

Two conclusions of the present work are that repeated measurements of blood count parameters during the day may improve diagnostic interpretation (e. g. leucocytosis?) and can reflect a complex diurnal interaction with the hormonal system. Further projects shall elucidate the diagnostic role of diurnal variation of routine hematological characteristics in disease, particularly in affective disorders where diurnal controls of DBC parameters may support or even replace cortisol measurements or a suppression test.

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