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Components of biological, including seasonal, variation in hematological measurements and plasma fibrinogen concentrations in normal humans

M. Maes, S. Scharpé[a], W. Cooreman[b], A. Wauters[c], H. Neels[c], R. Verkerk[d], F. De Meyer[d], P. D'Hondt[e], D. Peeters[e] and P. Cosyns[e]

University Department of Psychiatry, AZ Stuivenberg, 267 Lange Beeldekensstraat, B-2060 Antwerp (Belgium), [a]Department of Medical Biochemistry, University of Antwerp, [b]Laboratory of Clinical Biology, St-Augustinus Ziekenhuis, Antwerp, [c]Laboratory of Clinical Biology, Middelheim Ziekenhuis, Antwerp, [d]Royal Meterological Institute, Brussels, [e]University Hospital of Antwerp (Belgium)
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Abstract. This study has been carried out in order to examine the components of biological and, in particular, seasonal variation in hematologic measurements in normal humans. Toward this end, 26 normal volunteers had monthly blood samplings during one calendar year for determination of number of red blood cells (RBC) and platelets, hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), MC Hb (MCH), MC Hb concentration (MCHC), RBC distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), and plasma fibrinogen concentrations. The data were analyzed by means of spectral analyses of a group of time series or a single time series, and by means of repeated measures analyses of variance. Most of the hematologic variables show seasonal rhythms, such as annual rhythms or harmonics, which are expressed as a group phenomenon. An important part of the variance (> 15%) in Ht, MCV, MCH, MCHC, RDW, number of platelets, MPV and plasma fibrinogen was explained by a yearly variation. The peak-trough differences (expressed as a percentage of the mean) in the yearly variations in number of RBC, Ht, MCV, MCH, MCHC and RDW were very low (all <8.5%). Number of platelets (14.4%) and plasma fibrinogen values (28%) showed a high-amplitude yearly variation. All hematological variables, except MCHC, show a high interindividual variability which exceeds by far the intraindividual variability.
Key words. Seasonality; annual rhythms; hematology; red blood cells; platelets; fibrinogen; biological variation; coefficient of variation; index of individuality.

A multifrequency time structure has been observed at several levels of the hematopoietic system. Diurnal, infradian and ultradian periodicities may occur in the proliferation and maturation of hematopoietic precursors in the bone marrow, the release of cells from the bone marrow, plasma volume and counts of red blood cells (RBC) and platelets, and destruction and removal of those cells[1]. Significant circadian rhythms are observed in the number of circulating RBC, hemoglobin (Hb) concentrations, hematocrit (Ht)[2,3], number of circulating platelets[2], and plasma fibrinogen concentrations[3]. It is thought that the above circadian rhythms in hematologic variables may contribute to the circadian variation in the incidence of thromboembolic disorders, such as cardiac death, cerebral and myocardial infarct[2,3].

Comparatively few studies have focused on the seasonal variation in hematologic variables, probably due to the practical limitations of obtaining multiple samples over a long period of time[4]. A decrease in Ht in the summer was observed by Rocker et al.[5] Touitou et al.[6] found that seasonal changes in number of RBC, Ht and Hb were more pronounced in elderly (mean = ±76 years) than in younger (mean = 24.0 ± 3.9) persons. Several research groups have also observed a seasonal variation in multipotent stem cells in mice[4]. Lower temperatures have been shown to increase platelet count, the plateletcrit (PCT), number of peripheral RBC[7] and fibrinolytic activity[8]. Two studies have detected low-amplitude circaseptan rhythms in the number of circulating RBC, Hb and Ht[9,10]. Morley[11] found circatrigintan rhythmicalities in the number of peripheral platelets in 4 of 11 normal persons.

Interestingly, a significant seasonal pattern in thromboembolic-ischemic disorders has been demonstrated. Coronary and cerebral thrombosis and peripheral embolism[12–15] and fatal pulmonary thromboembolism[16] show a true seasonality with peaks in early or late winter and troughs in summer. It is hypothesized that seasonal variation in plasma fibrinogen, and number of platelets and RBC may result in changes in the hemostatic system, including clotting and flow, which, in turn, may alter the resistance/susceptibility to thromboembolic disorders[7,8,16].

The present study has been carried out in order to investigate the components of biological variation and, in particular, seasonal variation in hematologic variables, such as number of RBC, Hb, Ht, mean corpuscu-

lar volume (MCV), MC Hb (MCH), MC Hb concentration (MCHC), red cell distribution width (RDW), number of platelets, mean platelet volume (MPV), PTC, platelet distribution width (PDW), and plasma fibrinogen levels.

Subjects and methods

Subjects. The geographical coordinates for this study were 51.2°N and 4.5°E around Antwerp City, Belgium. Caucasian normal volunteers were selected to participate in the present study. Inclusion criteria were the following: a) Physically and psychologically healthy persons. The subjects were screened for past and current history of psychiatric disorders and personality disorders by means of the Semi-Structured Clinical Interview of the DSM-III-R[17,18]. All subjects were free from psychiatric or personality disorders. Subjects with anxiety and major psychological stress did not participate in this study. All normal volunteers were free of medical (e.g. endocrine, immune, metabolic) disorders, and of any drugs (including anticonceptiva); b) The willingness to have monthly blood samplings done; c) Living and working in an area <30 km from the Meteorological Station, Deurne, Antwerp, Belgium; d) A stable, settled life-style. Subjects regularly taking international flights and subjects who travel a lot, such as commercial travellers, were not included in this study. e) Normal blood tests, such as complete blood count, blood urea nitrogen, liver enzymes (SGPT, SGOT, γGT), hemoglobin, hematocrit, and thyroid function (basal thyroid secreting hormone, free thyroxine, and free triiodothyronine); f) Women of childbearing age were included if they were willing not to become pregnant during the study. Exclusionary criteria were the following: a) subjects with drug (alcohol, and any other drugs of dependence) use or abuse; b) Acquired immunodeficiency syndrome; c) Premenstrual tension syndrome; d) Tobacco use of more than five cigarettes a day; e) The occurrence of a new medical illness during the study, except a common cold or angina; f) Use of any new medical drugs during the study span, except occasional use of an over-the-counter drug such as aspirin; g) The occurrence of important negative life events during the study span (e.g. death of a parent, spouse). Two medical doctors (DP or PD, who were psychiatric residents and research assistants at that time) checked the medical and psychological condition of the normal volunteers every four weeks. Subjects were not allowed to spend more than one week in a geographic area other than the province Antwerp and to travel outside an area of more than 250 km from Antwerp city. After a vacation in another area, blood was sampled at least seven days after returning to Antwerp. Use of over-the-counter drugs, such as aspirin, was prohibited for at least two days prior to blood sampling.

Twenty-six normal persons (complying with inclusion and exclusion criteria) were selected to participate in this study. The major reason for refusal to participate in these studies was the restriction on travelling outside an area of more than 250 km from Antwerp city. There were 13 men and 13 women, mean age (SD) was 38.7 (13.4) years. The volunteers had the socio-economic status of the middle-class Belgian population, and a mean, net monthly income between \$1,500 and \$2,500. Subjects were all urbanized persons with a comparable rest-activity schedule. They were known to one of the authors and gave oral informed consent to participate in this study. The study span extended from December 11, 1991 until December 24, 1992. Sixteen subjects had their first blood sampling in December 1991, the others in January 1992.

Seasons were defined by their respective solstices and equinoxes, i.e. winter: December 21–March 20; spring: March 21–June 20; summer: June 21–September 20; and fall: September 21–December 20. Cross-seasons were defiened as the periods between: a) November 6–February 5; b) February 6–May 5; c) May 6–August 5; and d) August 6–November 5.

Methods. Blood collections were always performed in standardized conditions in order to minimize sources of preanalytical variation. An intravenous cannula was inserted at 08.00 h (± 30 min) in the antecubital vein of the subjects after an overnight fast. 65 mL of blood was taken during a period of 30 min. Each subject was always sampled by the same investigators (DP or PD). Each subject had 12 consecutive monthly blood samplings. In premenopausal females, blood samplings were carried out 5–10 days after the first day of the menstrual cycle. There were, during the study period, 59 days on which blood was sampled. The assays were performed on fresh blood. 250 µL of EDTA blood was used for assay of RBC and platelet parameters; 100 µL of citrated plasma was used for assay of fibrinogen. Other variables were assayed on the remaining serum/plasma fractions and the results will be published separately. RBC and platelet parameters were determined by means of a Coulter STKS fully automated total blood cell counter. Three parameters of the sample were simultaneously measured in the flow cell, i.e. volume, radiofrequency conduction and light scatter. Hb was determined by means of the cyanmethemoglobin method using a dilution of blood in a solution containing potassium cyanide and ferricyanide. Fibrinogen was determined by means of fixed time kinetic nephelometry for immunoprecipitation using the Behring Nephelometer (Behringwerke AG, Marburg/Lahn, Germany).

Statistics. Repeated measures analyses of variance (ANOVAs) were used to investigate interindividual variability with gender and age (<35 years versus ≥ 35 years) effects, intraindividual variability with seasonal,

Table 1. Seasonal differences in hematological variables in 26 normal controls.

Variables	Mean (SD)				ANOVAs*			Dunn Tests**		
	Fall (1)	Winter (2)	Spring (3)	Summer (4)	F-statistic	df	P-value	Contrasts	T-statistic	p-value
RBC (10E9/μL)	4.65(0.40)	4.75(0.42)	4.72(0.40)	4.65(0.40)	3.4	3/263	0.02	(1,4) vs (2,3)	3.8	0.0004
Hb (g/dL)	14.49(0.98)	14.41(0.95)	14.25(0.78)	14.33(0.86)	5.0	3/263	0.003	(1) vs (3)	2.5	0.01
Ht (%)	42.4(2.9)	42.8(2.9)	42.8(2.4)	42.4(2.7)	0.4	3/263	0.7	-	-	-
MCV (fL)	91.3(4.1)	90.3(4.7)	91.0(4.8)	91.3(4.6)	10.0	3/263	$<10^{-4}$	(2) vs (1,3,4)	5.4	$<10^{-4}$
MCH (pg)	31.2(1.5)	30.4(1.7)	30.3(1.8)	30.9(1.7)	43.2	3/263	$<10^{-4}$	(1) vs (2,3,4)	9.1	$<10^{-4}$
								(4) vs (2,3)	7.3	$<10^{-4}$
MCHC (g/dL)	34.2(0.6)	33.7(0.7)	33.3(0.7)	33.8(0.8)	23.2	3/263	$<10^{-4}$	(1) vs (2,3,4)	6.5	$<10^{-4}$
								(3) vs (2,4)	4.9	$<10^{-4}$
RDW (CV%)	12.83(0.88)	12.67(0.75)	12.91(0.97)	12.94(0.89)	5.4	3/263	0.002	(2) vs (1,3,4)	3.7	0.0005
Thrombocytes (10E3/μL)	270(67)	294(75)	270(75)	277(71)	14.8	3/263	$<10^{-4}$	(2) vs (1,3,4)	6.4	$<10^{-4}$
MPV (fL)	8.50(0.75)	8.20(0.70)	8.58(0.74)	8.54(0.76)	33.9	3/263	$<10^{-4}$	(2) vs (1,3,4)	10.0	$<10^{-4}$
PCT (%)	0.226(0.045)	0.238(0.050)	0.228(0.052)	0.233(0.047)	5.6	3/263	0.001	(2,4) vs (1,3)	3.6	0.0007
PDW (CV%)	16.07(0.46)	16.02(0.51)	16.13(0.48)	16.03(0.50)	1.0	3/263	0.4	-	-	-
Fibrinogen (mg/dL)	377(78)	368(92)	400(86)	376(73)	5.7	3/260	0.001	(3) vs (1,2,4)	3.7	0.0006

*Results of repeated measures analyses of variance. **Significance was set at $p = 0.007$.

Table 2. Results of cosinor analyses.

Rhythms	Variables	F-statistic	p-value
Annual	RBC number	8.3	0.0006
	Hb	4.4	0.01
	MCV	16.4	$<10^{-4}$
	MCH	65.8	$<10^{-4}$
	MCHC	27.2	$<10^{-4}$
	RDW	14.3	$<10^{-4}$
	platelet number	10.5	0.0001
	MPV	30.6	$<10^{-4}$
Semiannual	MCV	6.0	0.003
	MCHC	3.6	0.03
	platelet number	7.4	0.001
	MPV	13.0	$<10^{-4}$
Trimensual	RBC number	7.0	0.001
	Ht	10.0	0.0002
	MCH	8.6	0.0005
	MCHC	15.0	$<10^{-4}$
	RDW	3.7	0.02
	MPV	5.1	0.007
	PDW	3.4	0.03
Tetramensual	MCV	10.6	0.0001
	MCH	9.5	0.0002
	RDW	3.2	0.04
	platelet number	5.0	0.008
	PCT	5.0	0.007

Single cosinor analyses were carried out on the pooled time series of the normalized hematologic data with fitting of annual rhythms and harmonics, i.e. semiannual, trimensual and tetramensual rhythms.

cross-seasonal or monthly effects, and two- or three-way interactions between time × sex, time × age, and time × age × sex. Multiple *a priori* comparisons among treatment means (e.g. seasons) were checked by means of the Dunn test.

Rhythometry in time series was ascertained by means of least squares spectral analysis of a single time series or a group of time series and by means of multiple regression and cosinor fit analysis[19]. For a comprehensive review of the theoretical backgrounds of the procedures employed in the present study, the reader is referred to previous publications of one of the authors[19]. The statistical methods employed here and described by DeMeyer[19] are, in part, based on previous theories[20–23]. In short, spectral analysis searches for hidden periodicities in one or more time series on a probabilistic basis and allows an increased scanning of the whole frequency range. The significant rhythms are identified by relative sharp peaks rising above a continuous background. The only guarantee that these peaks correspond to real physical mechanisms is a significant signal-to-noise ratio in the spectral peaks. F-statistics are generated as a measure of the signal-to-noise ratio for each of the rhythms investigated and are listed in a periodogram or F-spectrum. In the present study, 100 frequencies (rhythms) were scanned in a range between 2 and 366 days. Group spectral analyses were carried out on the time series of the hematological values in the 26 subjects. The between + within F spectra were interpreted to make inferences on common rhythms expressed at the population level. Spectral analyses of single time series were performed on the pooled time series of the healthy volunteers after normalization of the hematological data relative to the yearly mean of the monthly measurements in each of the subjects (this eliminates all interindividual variability in the data).

All significant rhythms that might exist in the time series were determined by means of peak F-values in the F-spectra. These rhythms were used to compute a multiple component model: the total amount of variance in the hematological data (i.e. the dependent variables),

which was explained by the significant rhythms (i.e. the explanatory variables) was computed by means of multiple regression analysis[24,25]. The predicted values were used as an index of the estimated cyclic signal in the time series[24,25]. The results of the regression analyses were checked for autocorrelation by means of the Durbin-Watson statistic[26]. Thus, our procedure allows to delineate a multiple component model with estimates of orthophase (peak value) and range from low to peak values in a time series with different rhythmic components, as well as the relative contribution of each of the significant rhythms in explaining the variance in the raw data.

The total variability (CVt), total interindividual (CVtg; i.e. analytical interassay + interindividual), and intraindividual (CVti; i.e. analytical interassay + intraindividual) variability were derived by means of ANOVAs and expressed as coefficients of variation[27–30]. The intraindividual CVi and interindividual CVg values were calculated from the CVti and CVtg values using the formula: $CVt^2 = CVa^2 + CVg^2 + CVi^2$ where CVa is the analytical CV[27–30]. The index of individuality (I-index), i.e. CVi/CVg, was computed in order to evaluate the usefulness of conventional population-based reference values[28,30].

Results

Results of ANOVAs. Table 1 lists the results of repeated measures ANOVAs, which considered the seasons as repeated measures (complete results on cross-seasonal and monthly differences can be requested from the authors). The df in the denominator was determined by means of the number of subjects ($n = 26$), the replicated measurements in each subject (i.e. three monthly measurements in each season) and the missing values ($n = 20$ for all variables, except for fibrinogen: $n = 23$). The ANOVAs show that seasonal differences are present in most hematological variables, except in Ht and PDW. Although the seasonal (and cross-seasonal) differences in Ht were not significant, there were significant monthly differences ($F = 3.1$, $df = 11/255$, $p = 0.001$). There were no significant cross-seasonal or monthly differences in PDW. There were no significant season × sex, season × age (<35 years versus ≥35 years) and season × age × sex interactions for any of the variables measured.

Results of spectral and cosinor analyses. Group spectral analyses showed significnat ($p < 0.05$) annual rhythms in MCV, MCH, MCHC, RDW, number of platelets, MPV and fibrinogen; semiannual rhythms in Ht, MCV, number of platelets, MPV and fibrinogen; trimensual rhythms in MCH, MCHC, RDW, and MPV; and tetramensual rhythms in MCV, MCH, and PCT. Table 2 lists the results of cosinor analyses carried out on the pooled time series of the normalized hematologic data. Significant annual rhythms and/or harmonics were detected in most variables.

Results of regression analyses. Table 3 shows the outcome of 12 multiple regression analyses with the normalized hematological data as dependent variables and the significant rhythms as explanatory variables. The df in the denominator was determined by the number of measurements (312 measurements minus the missing values and outliers) and the number of significant rhythms (2 df per rhythm). For example, it was found

Table 3. Results of multiple regression analyses of hematologic data and plasma fibrinogen on significant rhythms.

Dependent variables	Significant rhythms in days (with exact p-value of regression coefficients)	R^2 (%)	F-value	df	p-value
RBC	366 ($<10^{-4}$); 97 (0.001); 39 (0.01)	13.8	7.6	6/284	$<10^{-4}$
Hb	293 (0.01); 112 (0.01); 64 (0.04)	8.8	4.6	6/285	0.0003
Ht	9 ($<10^{-4}$); 91 (0.0003); 144 (0.0003)	16.3	9.2	6/285	$<10^{-4}$
MCV	148 ($<10^{-4}$); 366 ($<10^{-4}$); 24 (0.003)	26.7	17.2	6/284	$<10^{-4}$
MCH	366 ($<10^{-4}$); 132 ($<10^{-4}$); 54 ($<10^{-4}$); 49 (0.004) 13 (0.009); 89 (0.03); 27 (0.06)	54.4	23.5	14/276	$<10^{-4}$
MCHC	133 ($<10^{-4}$); 366 ($<10^{-4}$); 91 ($<10^{-4}$); 49 ($<10^{-4}$) 64 ($<10^{-4}$); 20 ($<10^{-4}$); 27 (0.0002); 9 (0.001)	64.8	31.6	16/275	$<10^{-4}$
RDW	366 ($<10^{-4}$); 78 (0.001); 49 (0.002); 162 (0.09)	21.4	10.0	8/283	$<10^{-4}$
Platelets	366 ($<10^{-4}$); 220 ($<10^{-4}$); 126 (0.05)	18.9	11.1	6/285	$<10^{-4}$
MPV	366 ($<10^{-4}$); 200 ($<10^{-4}$); 86 ($<10^{-4}$); 122 (0.05)	36.9	20.7	8/283	$<10^{-4}$
PCT	121 (0.009); 366 (0.02); 194 (0.03)	7.9	4.1	6/285	0.0009
PDW	220 (0.03); 93 (0.03)	4.7	3.5	4/286	0.008
Fibrinogen	38 ($<10^{-4}$); 257 ($<10^{-4}$); 49 (0.0005)	25.2	15.9	6/283	$<10^{-4}$

Listed are the results of 12 regression analyses with the pooled time series of the hematologic data normalized to the mean as dependent variables and the significant rhythms (in days) as explanatory variables. The Durbin-Watson statistic was not significant in these regressions.

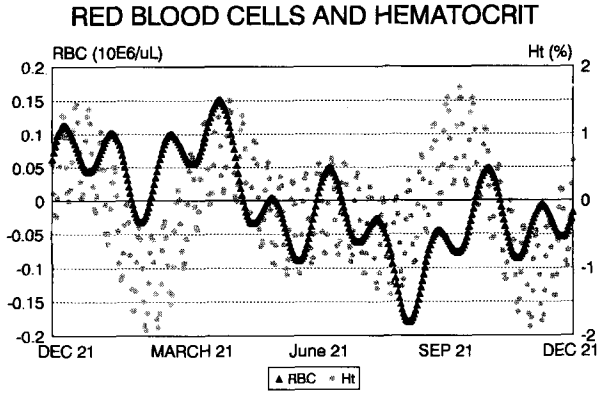

Figure 1. Seasonal variation in number of red blood cells and hematocrit. This chronogram shows the cyclic signal subtracted from the raw data by means of spectral analysis performed on the pooled time series of the data normalized relative to the yearly mean values in each subject.

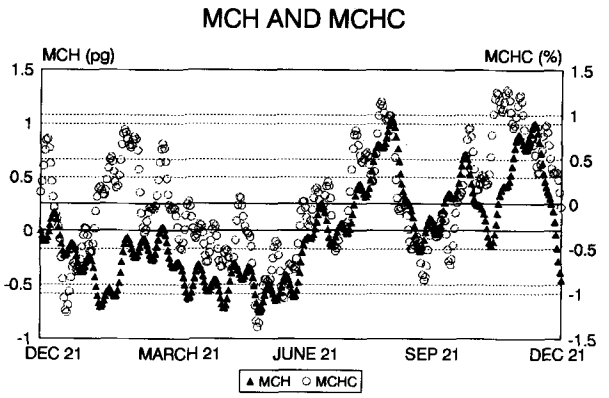

Figure 2. Seasonal variation in MCH and MCHC. This chronogram shows the cyclic signal subtracted from the raw MCH and MCHC data by means of spectral analysis performed on the pooled time series of the MCH and MCHC data normalized relative to the yearly means.

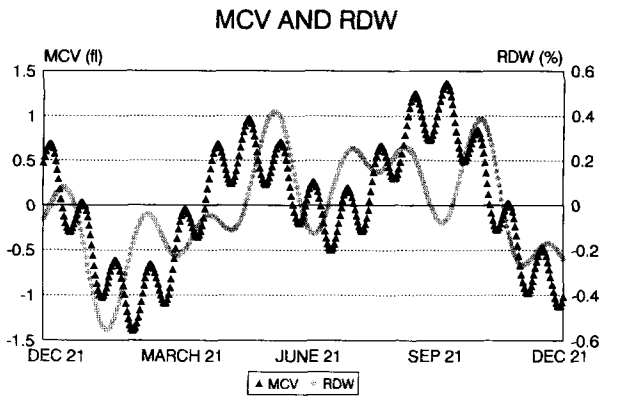

Figure 3. Seasonal variation in MCV and RDW. This chronogram shows the cyclic signal subtracted from the raw data by means of spectral analysis on the pooled time series of the MCV and RDW data normalized relative to the yearly mean values.

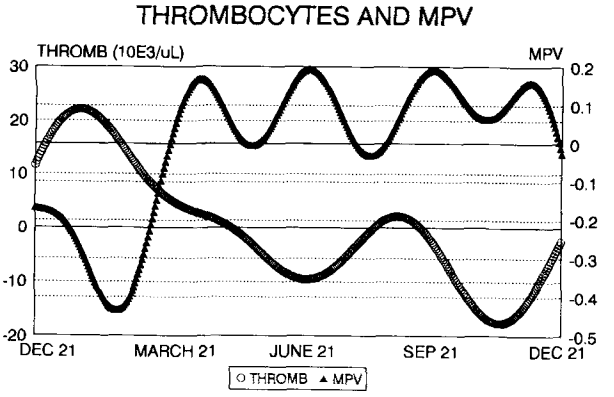

Figure 4. Seasonal variation in number of platelets and MPV. This chronogram shows the cyclic signal subtracted from the raw data by means of spectral analysis performed on the pooled time series of the data normalized relative to the yearly mean values in each subject.

that 13.8% of the variance in number of RBC was determined by three rhythms, i.e. annual (366 days), trimensual (97 days) and circamensual (39 days) rhythms; the significance of the regression coefficients shows that the annual rhythm was the most significant in explaining the variance in number of RBC. Seasonal rhythms and higher frequency rhythms explained an important part (>20%) of the variability in MCV, MCH, MCHC, RDW, MPV and fibrinogen, a moderate part (10–20%) in Ht, number of RBC and platelets, and a small part (<10%) in Hb, PCT and PDW.

Figures 1–5 show the cyclic signals in the various hematological variables. Figure 1 shows the chronograms of number of RBC and Ht. There were significant time-relationships between both factors (pooled $r = 0.92$, $p < 10^{-4}$). The time series of Hb values were significantly correlated with those of RBC (pooled $r = 0.84$, $p < 10^{-4}$) and Ht (pooled $r = 0.85$, $p < 10^{-4}$). Figure 2 shows the chronograms of MCH and MCHC. Both variables show significant time-relationships (pooled $r = 0.73$, $p < 10^{-4}$). Figure 3 shows the cyclic signals in the MCV and RDW values. There was a weak but significant relationship between both time series (pooled $r = 0.20$, $p = 0.0008$). In addition, significant relationships between the time series of MCV and MCH (pooled $r = 0.34$, $p < 10^{-4}$), MCV and MCHC (pooled $r = -0.39$, $p < 10^{-4}$), and MCHC and RDW (pooled $r = -0.12$, $p = 0.03$) were found. Figure 4 shows the cyclic signal in number of platelets and MPV. There was a significant and negative correlation between both time series (pooled $r = -0.43$, $p < 10^{-4}$). Figure 5 shows the cyclic signal in number of platelets and plasma fibrinogen. There were significant time-relationships between the time series of number of platelets and PCT ($r = 0.92$, $p < 10^{-4}$) and PDW ($r = -0.26$, $p < 10^{-4}$). Figure 6 shows, as an example, the raw MCH data (normalized with reference to the yearly mean in each subject) as well as the cyclic signal sub-

tracted from the raw data by means of spectral analysis in combination with multiple regression analysis.

Components of biological variation. Table 4 lists the CVt, CVg, CVi and CV values due to monthly differences (CVm), the I-index, and the peak-trough differences in the cyclic signals displayed in Figures 1–5 and expressed as a percentage of the mean. No CVa values were available for PCT and PDW; peak-trough differences in the yearly variation were not computed for these two variables and Hb, because the percentage of intraindividual variability explained by yearly variation was too low, i.e. $<10\%$.

The analytical interassay CVa values obtained were as follows: RBC: 0.4%; Hb: 0.6%; Ht: 0.8%; MCV: 0.6%; MCH: 0.7%; MCHC: 1.0%; RDW: 1.4%; number of platelets: 1.7%; and MPV: 1.4%; and fibrinogen: 5%. Since men had significantly higher numbers of RBC ($F = 14.9$, $df = 1/24$, $p = 0.001$), Hb ($F = 39.6$, $df = 1/24$, $p = 0.00002$) and Ht ($F = 29.9$, $df = 1/24$, $p = 0.00006$) values than women, and women had higher plasma fibrinogen values ($F = 9.1$, $df = 1/24$, $p = 0.006$), we have adjusted the interindividual variability (CVg) for gender.

The total variability in most variables, except number of platelets and fibrinogen, was very low (i.e. $<10\%$). The intraindividual (CVi) (all $p < 0.001$) and monthly variability (CVm) (all $p < 0.05$) were significantly greater than the analytical interassay variability (CVa). This shows that the analytical imprecision of our assays is small enough to detect the variability due to intraindividual and monthly differences. The interindividual variability (CVg) in number of RBC and platelets, MCV, MCH, RDW, and MPV was higher than the intraindividual variability (CVi). Accordingly, these variables showed a high degree of individuality (low I-index). The intraindividual variability (CVi) in MCHC, on the other hand, was higher than the interindividual variability (CVg) and, consequently, MCHC showed a higher I-index. Consequently, population based reference ranges are of interest for MCHC since the I-index is greater than 1.4[29,30]. Population-based reference ranges are of more limited use for the interpretation of the other variables since the I-index is lower than 1.2 and even lower than 0.6 for MCV, MCH, RDW, number of platelets and MPV[29,30]. Moreover, the finding that the variability due to monthly differences (CVm) in MCHC is greater than the interindividual variability (CVg) suggests that the population-based reference ranges for MCHC should be adjusted for time (e.g. seasons). The time-adjusted reference ranges for MCHC in the four seasons can be deduced from the results in table 1.

The seasonal peak-trough differences expressed as a percentage of the mean were low for most variables (i.e. $<10\%$), except for number of platelets and plasma fibrinogen which showed a high-amplitude yearly variation.

Discussion

The major finding of this study is that various hematological measures, such as RBC/Hb- and platelet-related factors and plasma fibrinogen levels, show a significant yearly variation in normal humans. The findings will now be discussed.

Number of RBC, Hb, Ht, MCV, MCH, MCHC, and RDW show multiple seasonal (e.g. annual, semiannual, trimensual) and higher frequency rhythms (e.g. 24–29 days) expressed as a group phenomenon. It was found that an important part of the variance in MCHC (i.e. 64.8%), MCH (i.e. 54.4%), MCV (i.e. 26.7%), but also in number of RBC (i.e. 13.8%), and Ht (16.3%) was explained by common rhythms. Nevertheless, the amplitude of the yearly variation in these variables is very low, i.e. only 3.0% to 8.5% of the mean values. This study found a higher number of circulating RBC in December–March than in June–December. Ht values

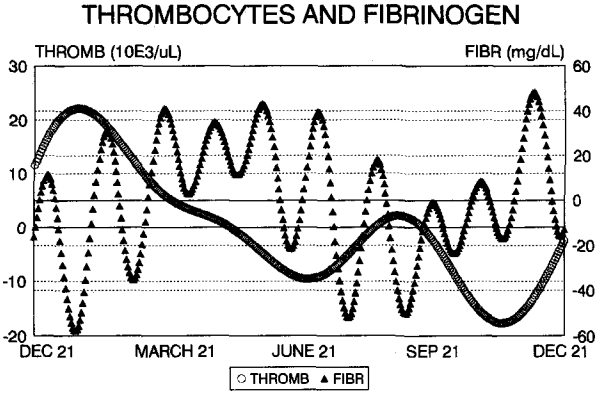

Figure 5. Seasonal variation in number of platelets and fibrinogen. This chronogram shows the cyclic signal subtracted from the raw data by means of spectral analysis performed on the pooled time series of the data normalized relative to the yearly mean values in each subject.

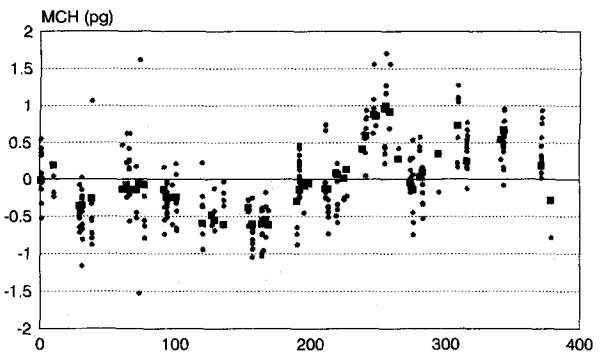

Figure 6. Time series (in days) of the raw MCH data (grey circles) and the cyclic signal subtracted from the raw data by means of spectral and multiple regression analysis (filled squares).

peaked in December, April and October with lows in March and August. Rocker et al.[5] found that Ht was lower during summer. Other authors observed that RBC count and whole blood viscosity increased at low temperatures[7]. In a four-time point study significant changes in number of RBC, Hb and Ht were detected by Touitou et al.[6]. In that study, seasonality was significantly more pronounced in elderly (mean age: ± 76 years) than in young (mean age: 24.0 ± 3.9 years) persons. Since we did not include persons older than 69 years and the mean age of the subjects was 38.7 ± 13.4 years, the results of our study are difficult to compare with those of Touitou et al.[6]. In any case, we were unable to find a significant difference in seasonality in any of the above hematological variables between younger (<35 years) and older (35–69 years) persons. Another major finding of this study is that number of platelets, MPV, and PCT show a significant yearly variation, expressed as a group phenomenon. In contrast to RBC/Hb-related variables, number of platelets show high-amplitude rhythms, i.e. the peak-trough difference is 14.4%. There was a significant time-relationship between the alterations in number of platelets and MPV; the former peaked in December–February, whereas the latter showed troughs in the same period. The increase in the number of platelets during the winter months, found in the present study, is in accordance with previous findings that low temperatures may induce increased platelet counts[7,31]. Circannual variations in biochemical properties of platelets have been reported, e.g. serotonin (5-HT) uptake in platelets, 5-HT contents in platelets, and ^{3}[H]imipramine or ^{3}[H]paroxetine binding to platelets[32–35].

The present study shows also that plasma fibrinogen concentrations exhibit a highly significant, high-amplitude (peak-trough difference is 28%) yearly variation, which is expressed as a group phenomenon. Peak values were observed in February–June and in December, whereas lows occurred in January and August–September. Previous research has shown that there is an inverse relationship between fibrinolytic activity and ambient temperature[8]. Other groups found a significant annual rhythm in plasma fibrinogen with an acrophase in February[36] or found higher plasma fibrinogen in colder than in warmer months[37]. However, the two last-mentioned studies are difficult to interpret since the delineation of 'seasonal variation' should not rely on differences between certain periods of the year or the detection of an annual rhythm by single cosinor. Least squares cosine spectral analysis in conjunction with cosinor fit and multiple regression analysis are most suited in order to examine 'seasonal' variation since these methods allow: 1) to identify all significant seasonal rhythms, i.e. annual rhythms as well as the harmonics, that may exist in a time series; 2) to determine the relative contribution of each rhythm to the variance in the time series; 3) to compute a multiple component model with estimates of orthophase (peak values) and the range of change from low to peak values in a time series with different rhythmic components; and 4) to check whether these rhythms are expressed as a group phenomenon.

New applications of clinical chemistry – which are important in a cost-conscious health care system – involve the use of yearly variations in the assessment of disease risk (e.g. cardiovascular disease) and the adjustment of population-based reference ranges for time (e.g. seasons) in so-called time-specified reference intervals or group chronodesms[38]. Thus, the high-amplitude rhythms detected here in some hematological variables, such as fibrinogen and number of platelets, may have diagnostic implications in that population-based reference ranges should be adjusted for seasonal effects. However, the results of the present study have shown that all variables, except MCHC, show a high degree of individuality, such that population-based reference ranges will be of low utility as diagnostic tools. In

Table 4. Estimates of total (CVt), interindividual (CVg), and intraindividual (CVi) biological variability, the variability due to monthly differences (CVm), the index of individuality (I-index) and the peak-trough (P-T) differences in seasonal variation.

Variables	CVt	CVg	CVi	CVm	I-index	P-T
RBC	8.6	7.7 (6.1)	4.0	1.5	0.65	7.03
Hb	6.2	5.0 (3.2)	3.6	1.0	1.14	-
Ht	6.5	5.0 (3.4)	4.0	1.6	1.15	8.46
MCV	5.0	4.7	1.4	0.8	0.30	3.01
MCH	5.6	5.2	2.0	1.5	0.38	5.37
MCHC	2.3	0.9	1.8	1.4	1.99	7.95
RDW	6.8	5.7	3.4	1.6	0.60	7.58
Platelets	26.1	24.3	9.1	4.0	0.37	14.39
MPV	8.8	8.1	3.4	2.2	0.42	7.65
Fibrinogen	21.9	16.2 (14.0)	13.5	6.5	0.96	27.96

All CV values are expressed as a percentage. The peak-trough (P-T) differences in the seasonal variation are expressed as a percentage of the mean. The CVg values were adjusted for gender (i.e. RBC, Hb, Ht and fibrinogen); the adjusted CVg values are shown between parentheses.

contrast, MCHC showed a low degree of individuality and, therefore, population-based reference ranges will be of greater utility. Moreover, it was found that the population-based reference ranges of MCHC should be adjusted for seasonal effects. It should be added that the CVi/CVg values obtained in the present study for the various variables, except MCHC, are in accordance with the CV values reported in previous papers (reviewed in 30). Fraser et al.[39] found that MCHC showed a CVi/CVg ratio of 0.6%/1.3% (=0.46) and 0.9%/1.7% (=0.53) in male and female subjects, respectively, whereas we found a ratio of 1.8%/0.9% (=1.99). This difference may, in part, be explained by the important yearly variation in MCHC reported here, which may not be observed in a study with a shorter study span.

At present, there is no exact information on the origin of the various seasonal rhythms in the hematological variables examined. It is thought that the circadian rhythms in hematologic variables are endogenously (i.e. genetically) determined and modulated by environmental factors ('zeitgebers'), such as climatic factors[1,40]. Future research should investigate 1) whether the seasonal variation in RBC and platelet characteristics is due to periodic alterations in proliferation or maturation of cell precursors in the bone marrow, their release from the bone marrow, their destruction or removal from the circulation; and 2) whether there is a time relation with alterations in messenger-molecules transducing environmental signals to the hematological system. For example, climate-induced changes in sympatho-adrenal-system activity could play a role in modulating platelet count and MPV[7].

It is tempting to hypothesize that there is a time-relationship between the seasonal variation in plasma fibrinogen, thrombocyte and RBC counts reported here, and the increase in hemostasis-related disorders in winter. Indeed, hermorrhagic stroke, peripheral embolism, and coronary and cerebral thrombosis show a peak incidence in winter[12–14,41–44]. Other findings suggest an inverse relationship between ambient temperature and the incidence of the above disorders[12,14,37,41]. Hence, it may be hypothesized that climate-induced alterations in biological rhythms, which modulate hemostasis (e.g. number of platelets, plasma fibrinogen), may be related to the seasonal changes in incidence of hemostasis-related disorders. Future chronoepidemiologic and biometeorologic research should examine whether there is an association between hemostasis-related disorders, seasonal variation in climate and thrombocytic or RBC characteristics and plasma fibrinogen concentrations. In addition, future chronopharmacologic studies of fibrinolytic/thrombolytic agents should investigate the yearly variation in their therapeutic effects in relation to the yearly variations in the hematologic variables reported here.

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