Seasonal changes in platelets, fibrinogen and factor VII in elderly people

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Abstract

Background: an increase in mean platelet volume and a decrease in platelet total have been reported following stroke and increased mean platelet volume in acute myocardial infarction has been shown to be predictive of mortality.
Objective: given the established seasonal variation in morbidity and mortality from cardiovascular disease and various risk factors for the disease, we explored the seasonal variation in mean platelet volume and platelet total.
Methods: we assessed levels of platelet count, platelet volume, fibrinogen, factor VII, core body and ambient temperatures in 54 healthy community dwelling elderly volunteers over a period of 1 year. We used cosinor rhythmometry to quantify and compare the seasonal rhythms.
Results: we found significant seasonal variation in fibrinogen, mean platelet volume and core body temperature all of which peaked synchronously in May/June, in a year with an atypically mild winter and hot summer. Platelet total and factor VII did not exhibit a seasonal rhythm.
Conclusions: we conclude that the synchrony between peak size of platelets and peak level of fibrinogen will significantly increase the likelihood of thrombotic events. These results provide further evidence of a seasonal pro-thrombotic state, which has a complex relationship with temperature.

Keywords: *thrombotic risk, season, platelets, fibrinogen, temperature*

Introduction

The increased morbidity and mortality from cardiovascular disease in winter months has been extensively reported [1]. Seasonal variation has been demonstrated in blood pressure [2–4] and in fibrinogen, a major cardiovascular risk factor [5–8]. However, little is known about how other haemostatic factors change with season. Factor VIIa levels have been shown to increase in winter months [7] while the present authors showed a seasonal change in protein C and antiplasmin [9]. There seems to exist a prothrombotic state during winter.

An increase in mean platelet volume (MPV) and a decrease in platelet count (PLT) have been reported following stroke [10] and elevated MPV has been noted in acute myocardial infarction (AMI) and to be predictive of subsequent death [11–13]. Although we originally reported that PLT did not vary seasonally [5] and this was subsequently confirmed by others [8], one group have reported an increase of 30% of a standard deviation in PLT in the coldest months [6]. In light of the changes in MPV following stroke and AMI, this area warranted

further investigation. In the present study, we investigated the seasonal variation in platelet size and count and the relationship of these variables to the clotting factors fibrinogen and Factor VII (FVII) and to core body and ambient temperatures.

Methods

Subjects

Measurements were carried out on monthly plasma samples obtained from 54 healthy elderly subjects (26 male, mean age 80.5 years, SE 0.8; 28 female, mean age 80.1 years, SE 0.8) recruited through general practices in Belfast, UK. Subjects were included in the study following an initial health assessment which excluded individuals with existing medical conditions or who were taking medications which may have affected the measurements of interest. All participants were reported well and living in the community. They were visited in their own homes at

Table 1. Annual rhythm parameters determined by the single and population-mean cosinor procedure

	Mesor (95% CI)	Amplitude	F-statistic	*P*	Seasonal variation	Acrophase (° from 0 where 0° = 1st Jan)	Time of peak (month)
Fibrinogen (g/l)	3.54 (3.37–3.72)	0.077	3.509	0.05	0.154	−165°	June
Factor VII (% standard)	122.57 (115.06–130.08)	3.980	0.276	–	–	−87°	–
PLT (e9/L)	228.69 (214.63–242.75)	2.326	0.788	–	–	−21°	–
MPV (fl)	8.869 (8.64–9.10)	0.071	3.324	0.05	0.142	−123°	May
Core temperature (°C)	35.95 (35.80–36.12)	0.349	6.622	0.01	0.698	−139°	May
Maximum ambient temp (°C)	13.50 (12.63–14.37)	6.828	78.602	<<0.0001	13.656	−221°	August
Minimum ambient temp (°C)	6.39 (5.64–7.14)	5.385	65.633	<<0.0001	10.770	−230°	August

monthly intervals for a period of 1 year, and a blood sample obtained at each visit. Core body temperature was measured using a Genius® tympanic thermometer (Kendall, New York, USA). To control for circadian variation in the measurements, all visits took place in the morning and subjects were sampled at the same time on each visit. The Meteorological Office supplied mean monthly ambient temperatures for Northern Ireland.

Blood

Venous blood samples were drawn using a 21-gauge needle and dispensed into bottles containing 3.8% tri-sodium citrate. Samples were transported to the laboratory at ambient temperature and plasma was separated from the cells within 3 hours of collection (normally within 1 hour) by centrifugation at $2000\,g$ for 20 minutes. Some fresh plasma was used immediately for fibrinogen determination using the Clauss assay [14] and the remainder was aliquotted and stored at −70 °C until required. The use of frozen plasma completely excluded assay drift. FVII was measured photometrically (Chromogenix, Sewden) in frozen plasma according to the test kit manufacturer's protocol. Platelet measurements were determined using a Coulter Blood Analyser as part of the routine of the Regional Haematology Laboratory, Belfast City Hospital. Both fibrinogen and FVII measurements were carried out by a single operator using the same instrumentation and reagents throughout. Fibrinogen tests were also standardised by the inclusion of a reference plasma in each assay. The coefficient of variation of the clotting times of the reference plasma at 1/10 dilution was 2.8%.

Data analysis

All blood measurements and core body temperatures were analysed for seasonal variation using the population-mean cosinor method [15–17]. These methods are statistically very powerful; when measurements are assigned more or less evenly along a full cycle of periodicity (in this case 1 year), 80% power can be reached to resolve the rhythmic structure of the data based on a relatively small sample ($k \geq 3$). Our sample of 54 subjects would greatly increase the power from 80%. Cosinor models are fitted to each individual's data for a particular variable. The resultant parameters are combined to give a population value namely: Mesor or rhythm-adjusted mean; Amplitude, the difference between the mesor and the highest or lowest value, the seasonal variation is therefore twice the amplitude; Acrophase or time of peak value in calendar months. Ambient temperatures were quantified using the single cosinor method. Statistical significance of each seasonal rhythm was determined by F-test ($F_{1-\alpha}(2, n-3)$) of the amplitude $A \neq 0$.

Results

Results of the single cosinor (ambient temperatures) and population-mean cosinor (blood measurements and core body temperature) are presented in Table 1. The actual mean levels of each blood measure ($n = 54$) are represented in Figure 1 and core body temperature in Figure 2. Significant seasonal rhythms were present for fibrinogen (amplitude 0.08 g/l, peak June, $P = 0.05$), and for MPV (amplitude 0.07 fl, peak May, $P = 0.05$) (Table 1, Figure 1a). The seasonal variation in PLT and FVII was not statistically significant (Table 1, Figure 1b). Measurements of core body temperature showed a statistically significant seasonal variation and a peak corresponding with those for ambient temperatures, fibrinogen and MPV (Table 1, Figure 2). Ambient temperatures were mild in the winter months and unusually hot during the summer period with a peak in August (Table 1, Figure 2). Outdoor mean maximum temperatures for the period of study were on average 1.2°C higher compared with a mean maximum temperature for the previous 16 years (Figure 3a). Mean ambient minimum temperature for the winter quarter of the study cycle (November, December, January, February) was one of the mildest in a 20-year period (Figure 3b).

Discussion

We report a seasonal variation in mean platelet volume, coinciding with the seasonal variation in plasma fibrinogen. This confirms the recent study carried out in the Netherlands by Maes and De Meyer [18] who reported seasonal rhythms in MPV and fibrinogen together with their association with climatic data. They propose that the seasonal rhythms observed in immune/haematologic variables may be entrained by the seasonal rhythms in
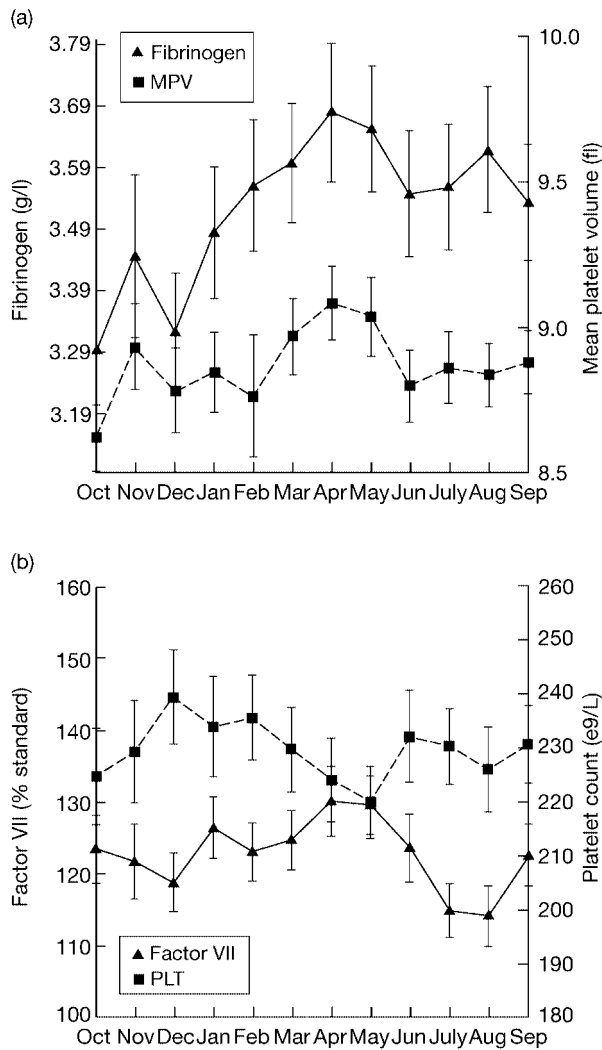
Figure 1. Monthly levels of fibrinogen, Factor VII, total platelet count and mean platelet volume (mean and standard error), *n* = 54. (a) Measures with significant seasonal variation (b) Measures without significant seasonal variation.

Figure 2. Monthly data for mean core body temperature (mean and standard error), *n* = 54 and minimum and maximum ambient temperatures.

Figure 3. (a) Mean maximum ambient temperature over 20 years for Northern Ireland and (b) mean minimum ambient temperatures for the winter quarters over the same 20-year period.

ambient temperature as suggested by our original report [5]. In the present study, the total platelet count did not exhibit a statistically significant seasonal variation; however it declined at the same time as the mean platelet volume increased. The current findings are interesting in light of those reported by O'Malley *et al.* [10] with respect to changes in these variables following acute stroke. A persisting rise in MPV and a reduction in PLT have been described in AMI [13]. Zeiger *et al.* [19] have recently confirmed this inverse relationship between PLT and MPV.

In the present study, we have again demonstrated a significant seasonal variation in fibrinogen levels. However, the levels of fibrinogen peaked later in the year, the maximum values occurring in June as opposed to the winter months. A similar finding was reported in a study carried out in Germany, where fibrinogen levels peaked in April [20]. In the present study, core body temperature also peaked in phase with fibrinogen and
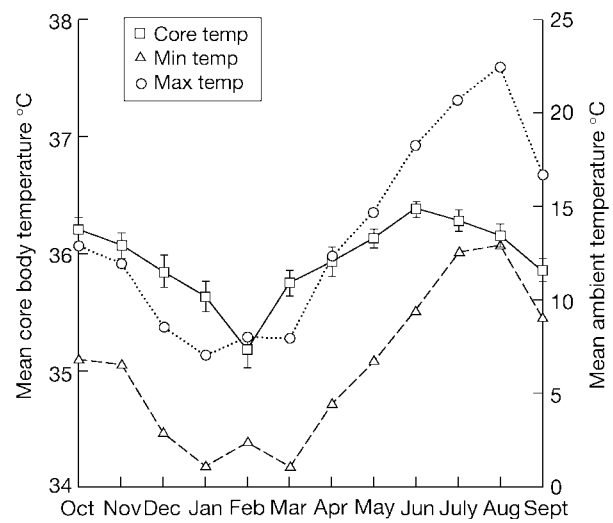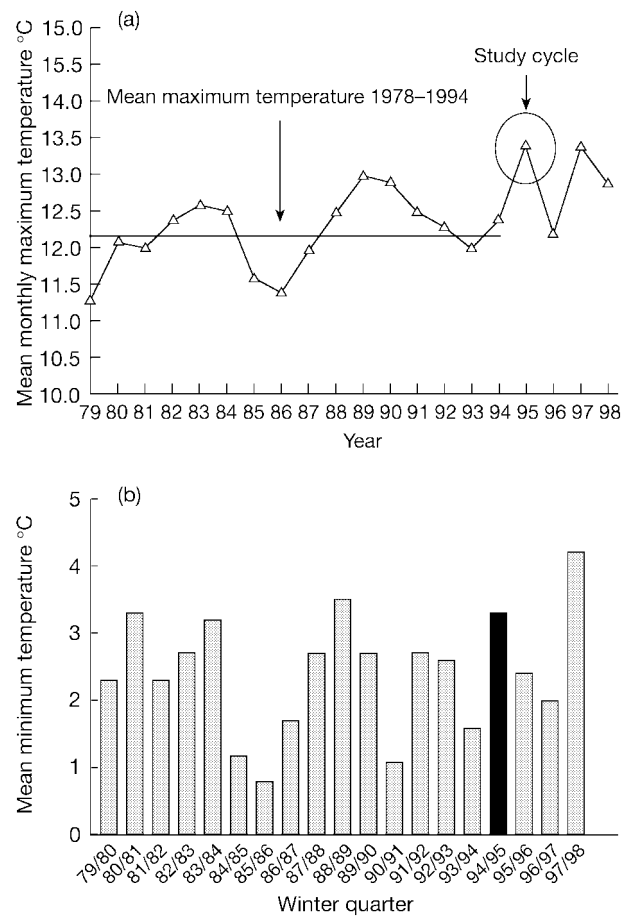
MPV. This is contrary to our original report where fibrinogen peaked when core body temperature was lowest [5]. However, during this investigation it may have been that core temperature did not fall sufficiently during the winter months to cause the normally observed increase in fibrinogen. The annual cycle investigated had a mild winter and atypically a very hot summer. It may be that although Belfast has a temperate climate, in this year, the response was similar to that reported in warmer countries where the peak cardiovascular mortality and prevalence is consistently observed in the hottest months [21–24]. A 'U' shaped response to ambient temperatures has been proposed for cardiovascular mortality, where deaths increase at extremes of physiologically 'comfortable' temperatures [25]. The mild winter quarter at the start of the cycle studied in this investigation may have resulted in a small winter peak while the persistently hot summer produced the larger response not normally seen in more typical summers. This might also suggest that the usual seasonal variations in fibrinogen and MPV are not related to winter infections. There was also no association with or seasonal variation in white cell count, C-reactive protein and neutrophil count which were also measured in this study (but not reported here).

Analysis of mortality data for myocardial infarction for the cycle presented here, October 1994–September 1995, revealed a peak occurring in mid-March, some 8–10 weeks before our peak levels of MPV, fibrinogen and core body temperature. It is possible that individuals susceptible to the increasing thrombotic state succumbed at an earlier time, while haemostatic factors continued to rise as seen in this study population. Elderly people are potentially more at risk from thrombotic events and we have shown their seasonal response in fibrinogen to be greater than in younger subjects [9]. However, these subjects have survived to an average age of 80 years without a thrombotic even occurring; therefore their haemostatic mechanisms have been successful. This perhaps supports the idea that more susceptible individuals have already suffered a fatal event.

FVII did not vary seasonally in the present study which is in contrast to the work of Woodhouse et al. [7] the only study to date to report a seasonal change in FVII. One possible reason for the discrepancy is the difference in methodology between the two studies. Woodhouse and colleagues employed a one stage clotting assay which is sensitive to levels of circulating activated FVII (FVIIa). The chromogenic assay employed in the present study measures total FVII without being influenced by FVIIa levels. This might suggest that total FVII does not vary with season but endogenous activated FVIIa does. This would be worthy of further investigation. The development of assays specific for FVIIa allows these measurements to be compared. Since circulating FVIIa levels are thought to be the real risk factor for cardiovascular disease, a seasonal change in FVIIa levels rather than total FVII would go further to explain the seasonal change in cardiovascular disease.

We have again demonstrated a seasonal variation in fibrinogen levels. However in this annual cycle the peak level occurred later in the year as opposed to the winter months. The cycle investigated had a mild winter and a very hot summer. This is the first report of a synchronous peak in MPV and fibrinogen using the population-mean cosinor method. Larger platelets are more reactive and produce more thrombotic factors. The synchrony found in this investigation between the peak size of platelets and the peak level of fibrinogen will significantly increase the likelihood of thrombotic events at this time in the annual cycle.

Key points

- There is a significant seasonal variation in fibrinogen, mean platelet volume and core body temperature, all of which reach a synchronous peak.
- This synchrony between peak level of fibrinogen and peak size of platelets will significantly increase the likelihood of thrombotic events.
- This seasonal pro-thrombotic state appears to have a complex relationship with ambient temperature.

Acknowledgements

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