

Circadian Rhythms of the Salivary Proliferation Markers CA130 and CA125 in Clinical Health

The hypothesis that a circadian rhythm of large amplitude of the tumor markers can be determined with saliva sampling at times of voiding by a clinically healthy woman was examined and validated. One of these markers, CA125 (1) is used in diagnosis and monitoring as well as prognosticating patients with gynecologic malignancies. Circulating CA125 is also elevated in benign conditions (7, 10, 11). In view of the inconsistency and/or the high noise levels of spontaneous CA125 variation in the serum of patient with an ovarian cancer, the search for a marker with a more prominent rhythm is of great interest. Saliva and urine are obvious candidates; saliva was examined herein since a circadian rhythm of large amplitude in CA125 (6, 8) and in CA130 was described in the saliva of a 72-year-old patient with a müllerian cancer. If CA125 of saliva also exhibits a circadian rhythm in clinical health, the characteristics of this rhythm in the absence of an overt cancer could perhaps serve by their alterations as indices of risk. EINHORN *et al.* (5) have reported a case with an increase in circulating CA125 occurring several months before the diagnosis of an ovarian cancer.

A clinically healthy woman, 28 years of age, collected saliva around the clock from April 10 to 21, at approximately 4-hour intervals during waking, with an occasional interruption of sleep for sampling. She was on a routine of wakefulness usually from 07:00 to 00:00 h. Samples were collected at least 1 hour after any intake of food or fluids, without rinsing of mouth. About 1 ml of it was frozen immediately below -25°C . Determinations of CA125 were done on samples from April 10 to 14 and of CA130 on all samples, from April 10 to 21, both by RIA (1-3, 12). Data series were analyzed by rhythmometric methods, notably by the single cosinor (9). Point-and-interval estimates are obtained for several characteristics. These parameters are the MESOR, the circadian double amplitude, and the acrophase. Conventional statistics consisting of the computation of a mean value, a standard error and ANOVA were also carried out.

CA125 and CA130 between 04:00 and 08:00 and to a lesser extent between 08:00 and 12:00, are elevated as compared to other times. When the analysis is performed on short series (04/10-14), the time effect is of borderline statistical significance. A one-way ANOVA finds a statistically significant time effect when data were $\log_{10}$ (CA125, $P = 0.034$; CA130, $P = 0.037$).

When the analyses are carried out for the longer series of CA130 (04/10-21), a circadian rhythm, estimated by single cosinor, is statistically highly significant. The circadian rhythm remains prominent, almost invariably a peak in salivary CA130 concentration occurs in the morning (table I). Moreover there is good agreement between the acrophases of CA125 and CA130 ($\approx 90^{\circ} \equiv 06:00$ a.m.). A circadian rhythm can be readily assessed for the case of CA130 once sufficient samples are available for analysis. The statistically significant time effect (circadian pattern of salivary CA130) is also validated by ANOVA.

The circadian rhythm documented for this clinically healthy woman (AP) resembles that found earlier for an ovarian cancer patient (EH) (4), with high values in the early morning and low values in the afternoon. Whereas acrophases occur approximately at the same circadian stage in both AP and EH, the MESOR and circadian amplitude are higher in EH. For AP, CA130 was found to be consistently lower as compared to CA125.

CHEN *et al.* (3) had proposed a cut-off value of 3000 U/ml for CA125 in saliva collected in the morning after an overnight fast and after a thorough rinsing of the mouth. The prominent circadian variation characterizing both markers should be taken into consideration for establishing critical time-specified thresholds for CA130. This stipulation (3) is a step in the right direction. "On awakening" may be a more precise time specification and may reduce uncertainties associated with failure to sleep, e. g., by a shift-worker, but whether, and to what extent sleep plays a role in a subject. Proliferation markers of saliva exhibit a circa-

Table I: Circadian profile of salivary CA125 and CA130 (U/ml) in health.

Circadian stage (h)	N	CA125 (04/10-14)	CA130 (04/10-14)	N	CA130 (04/10-21)
00:00-04:00	2	792 ± 332	688 ± 196	3	675 ± 114
04:00-08:00	2	3165 ± 949	2487 ± 717	7	3032 ± 529
08:00-12:00	5	1730 ± 874	1457 ± 714	11	1475 ± 452
12:00-16:00	5	557 ± 22	506 ± 46	13	580 ± 46
16:00-20:00	5	830 ± 207	728 ± 137	12	664 ± 66
20:00-24:00	4	784 ± 89	658 ± 50	15	462 ± 55
Overall	23	1158 ± 248 ^{a, d}	975 ± 196 ^{b, e}	61	1015 ± 144 ^{c, f}
MESOR ± SE		1301 ± 257	1085 ± 203		1195 ± 129
Amplitude ± SE		854 ± 385	658 ± 310		1020 ± 194
Acrophase (CI) ¹		-89° (-53, -148)	-92° (-55, -153)		-92° (-74, -115)

N = saliva samples in each circadian stage collected around the clock; MESOR = midline-estimating statistic of rhythm; rhythm adjusted mean; amplitude = half of the total predictable change within one cycle; acrophase = a measure of timing of overall high values recurring each cycle, expressed in negative degrees, with 360° = 24 h; 0° = 00:00 and the reference time chosen as local midnight for the case of circadian rhythms studied herein.

¹ For series of 23 data, 90 % confidence limits are given, whereas for series of 61 data, 95 % confidence limits are listed. P (ANOVA): ^a0.081, ^b0.099 and ^c < 0.001; P (Single cosinor) ^d0.090, ^e0.092 and ^f < 0.001.

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Key words: Tumor marker, CA125, CA130, Ovarian cancer, Saliva, Chronome, Circadian rhythm.

Palabras clave: Marcador tumoral, CA125, CA130, Saliva, Cáncer ovárico, Cronoma, Ritmo circadiano.

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