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Chronobiology in epidemiology and preventive medicine

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Summary. - Many of the known cardiovascular risk factors, such as hormones, metabolic parameters, lifestyle, blood pressure, fibrinogenesis, fibrinolytic activity, etc., and also the reactivity of the organism to stimuli, are periodical and therefore require adequate measures at specific times in appropriate frequencies. The presence of circadian, circannual or other wide frequency rhythms may lead to contradiction in the evaluation of a potential risk factor. Chronobiological analysis shows fine differences within a «risk variable», defining some characters which may imply the alteration of a parameter only at certain times. Periodical risk factors may therefore be foreseen and sometimes prevented. Moreover, chronobiological approach may show another peculiar relation between risk factors; this is the so-called chronorisk. The convergence, in certain periods of time, of the phases of various risk factors, may strengthen their combined value. On the other hand, the phases of the protective factors, in the same period of time, may contribute to reduce or strengthen this combined value. From this point of view combined risk, apparently unimportant, appears highly increased, as compared with the normal homeostatic value. At equal levels of risk factors, different distribution and amplitude of the phases implies great differences in final combined risk values. Acute heart attack has a daily, seasonal and perhaps ultradian rhythm; other cardiological events, such as angina pectoris and sudden death, have circadian rhythms. Also cerebral vascular events have daily, seasonal, weekly and monthly variations. The periodical variations of acute vascular events and of the related risk factors, near and far, implies many complex interrelations in timing of biological structures, which are still perceived by the physician only as «noise».

Key words: chronoepidemiology, chronorisk, preventive medicine.

Riassunto *(La cronobiologia in epidemiologia e medicina preventiva)*. - Molti fattori ritenuti di rischio quali ormoni, parametri metabolici, stile di vita, la pressione arteriosa, il fibrinogeno, l'attività fibrinolitica, ecc., come pure la reattività dell'organismo agli stimoli, sono periodici e pertanto richiedono adeguate misure tempo-specifiche in frequenze appropriate. La presenza di ritmi circadiani, circannuali o, in altri campi di frequenza, di grande ampiezza, può portare a risultati contraddittori nella valutazione di un potenziale fattore di rischio. L'indagine cronobiologica permette il rilievo di più fini differenze nell'ambito di una «variabile a rischio» definendone alcune caratteristiche che possono rivelare parametri alterati solo a determinati tempi. La periodicità, inoltre, implica come corollario la prevedibilità di un evento e, talvolta, la sua prevenzione. L'approccio cronobiologico può tuttavia mostrare un'altra potenziale, precipua caratteristica interrelazione tra più fattori di rischio: il cosiddetto *cronorischio*. La convergenza, in determinati periodi di tempo, delle fasi dei vari fattori di rischio ne potenzia il valore di combinazione che, a sua volta, può essere ridotto o ulteriormente potenziato se, nello stesso arco di tempo, coincidono o meno le fasi dei fattori protettivi. In questa luce il rischio combinato, apparentemente insignificante, risulta notevolmente aumentato rispetto alla comune ottica omeostatica. A parità di livelli, una diversa distribuzione delle fasi, una diversa entità dell'ampiezza comporta del rischio valori affatto differenti. L'infarto miocardico acuto risponde ad una cadenza diurna, stagionale e forse ultradiana; altre manifestazioni della *Passio Cardiaca*, quali l'angina e la morte improvvisa, hanno una localizzazione temporale circadiana. Anche gli eventi vascolari cerebrali sono sottoposti a variazioni giornaliere, stagionali, settimanali e mensili. La dimostrazione di periodicità a varia frequenza in eventi vascolari acuti e dei relativi fattori di rischio, alcuni lontani altri vicini, implica molteplici e complesse interrelazioni nel *timing* delle strutture biologiche che il medico di oggi percepisce ancora solo come «rumore».

Parole chiave: cronoepidemiologia, cronorischio, medicina preventiva.

Introduction

Some individuals may lead a long life on a purely genetic basis; again on a genetic basis or because of an undesiderable life style or as a result of a combination of factors, others are at risk.

Man is not only the result of his genetic order but also the product of external environment. Disorders caused by malnutrition and infection among underpriviliged people constitute the most publiced evidence of the role played by environmental factors in disease causation. But changes in the pattern of

disease that have occurred in prosperous countries during the past few decades, the so-called *onco-degenerative diseases*, provide just as convincing illustrations of this relationship. Civilization, life estrangement from natural conditions and cycles governing human evolution, threaten and interact with genetic structure, producing *states for a long time reversible, but potentially evolutive in morbid affections*.

Severe diseases and death in mature age are correlated with vascular events and tumors.

Clinical and experimental evidences indicate that the clinical phase, which is the symptoms phase, represents a very short (one third) duration of natural history of disease, being clinically silent a great part of the course.

This remark clearly challenges the early diagnosis and perhaps relegates it into the sphere of clinical mythes.

Thus, it is arised the need not only of an early and pre-clinical diagnosis, but, in oncodegenerative diseases, the prevention seems mandatary: «*An ounce of prevention is worth a pound of cure*».

Epidemiology and chronoepidemiology

All men are potentially liable to the same kinds of diseases and all derive benefits from the same kinds of medical care. Yet despite the fundamental biologic unity of mankind, each geographic area of human settlement, each type of society and each economic group is characterized by its own pattern of diseases and has special medical needs. We can define habits, characteristics, anomalies associated to a reasonable rise of disease susceptibility. These peculiarities include hereditary factors, life style, social and economic conditions, enviroment reactions, generically called «risk factors» by epidemiologic research.

The term is borrowed to insurance companies language but in medicine we define a «risk factor» as a statistical, positive (or negative) relationship between the presence of a factor and the symptoms of a disease or another clinical phenomenon.

It is characteristic of people who are still healthy, even if, unlike «normal» subjects, they are going to develop a disease, without a necessary implication of cause-effect relationship.

Besides, these «characteristics» can get either close to disease, or they can make it foreseeable after a long time.

Clinical-epidemiologic research has identified a quality of specific characteristics, distinguishing subjects who are going to be healthy from subjects who are going to fall ill, and among them the disorders they'll undergo.

Characteristics derive from observation of conditions provoking specific disease development and from research of reasons of a lower incidence in those subjects where we expected a higher one.

In clinic this kind of approach is more important: high risk subjects who don't develop the expected disease represent precious sources for holding possible protective factors.

At the same time subjects without «risk factors» who contract the disease are interesting too. This is the clinical concept of the *negative's* importance [1].

In this group without apparent risk the presence of unknown risk factors or incidental absence of defensive factors is possible: they show a higher vulnerability conditions.

From this point of view subjects can be divided in positive and negative as to a particular affection.

So, when subjects show positive and negative factors, the resulting risk could be represented by their algebric addition.

Infact several studies have demonstrated that the presence of more positive or aggressive risk factors gives a combination whose value is higher than the simple algebric addition of single values (*combined risk*); besides, prediction value of combined risk has a higher significant clinic equivalent.

When precised, risk value can be transferred from a statistical-probabilistic concept, to a subject and so considered in function of «predisposition».

The old concept of diathesis and its variations (intensity, polyvalency, heredity) of classical medicine, that is genotipic predisposition to one or determined diseases, is now revaluated but in terms resulting from interrelation of several factors and not only from genoma.

Many factors considered at risk for onco-degenerative disease as hormones, metabolic parameters, life style, arterial pressure, as well as organism reactivity to stimuli are periodic and ask for adequate time specific measures in appropriate frequencies [2]. The presence of rhythms can lead to contradictory results in a potential risk factor evaluation.

Chronobiology, by exploring and quantifying the time-depending rhythmic variations of human organism, that always occur, whether they are subjects of study or not, opens a new dimension for clinical and preventive medicine.

The large-scale introduction of a true time dimension into biology and medicine should depend very heavily on the advent of the computer, the time-microscope, that led to the recognition of the ubiquity of rhythms as the complement to cells, even when some of the rhythms were not apparent in noisy data. The periods of these rhythms can vary from a split second to seconds, to a few hours, as in endocrine functions (ultradian rhythms), to about 24 h as in the prominent frequency range (circadian) which can be found, almost ubiquitous, in all metabolic structures and physiological functions. Superimposed to circadian rhythms, there are longer period variations (infradian). These facts as yet are largely unexploited in preventive medicine. As long as they are unrecognized, circannual clinical chemical changes are dismissed as complicating matters.

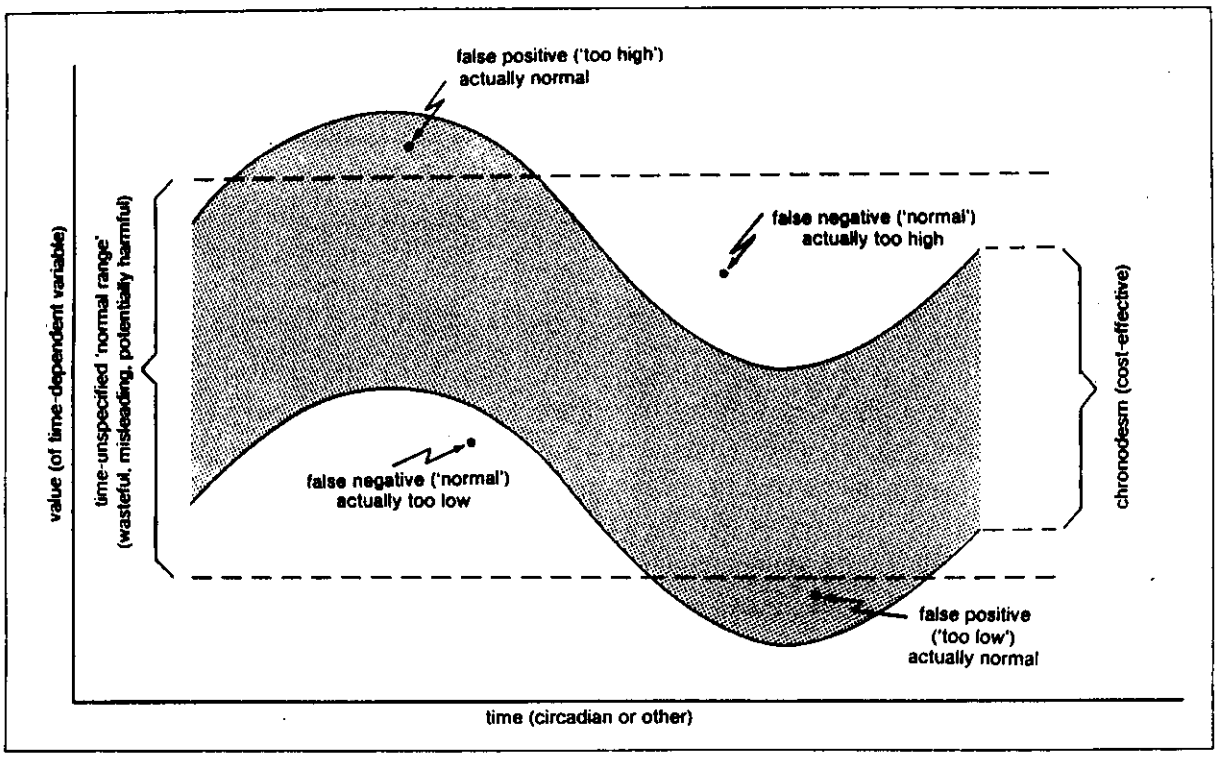

Fig. 1. - Abstract scheme emphasizing advantage of chronodesm over usual (time-unspecified) «normal range» in clinical diagnosis. (Reproduced with kind permission from [4]).

Further, many parameters show significant periodicities in several frequency domains, so that a biomedical value may partially be determined by the interaction of several rhythmic variations of different frequencies.

This network of numerous periodicities constitutes the so-called «temporal anatomy» [3].

The identification of a human complex timing structure asks for a redefinition of the concept of normalcy, as normality in internal and external time.

A clinical useful distinction considers rhythms with *great amplitude* and rhythms with *small amplitude* which can be only resolved with special time microscopic methods of statistical time series analyses.

Some rhythms of large amplitude may pose diagnostic problems: in this case the use of time qualified usual ranges, the so-called *chronodesms*, are fundamental and cost/effective. As emphasized at the outset, one important factor usually ignored in conventional «normal ranges» is the timing of a biomedical measurement in relation to biologic rhythms. In certain cases, reference intervals determined irrespective of time are much too broad and indiscriminate. The same value, when referred to an appropriate chronodesm, may in fact be too low at one time, quite «normal» at another time and too high at yet another time. In other words, a «normal» value in conventional terms may well be a false positive or a false negative, depending on when the measurement was made in relation to a rhythm in the variable concerned (Fig. 1).

With an adequate data base, several reference intervals, qualified as to time, have been established with different methods and in function of circadian, weekly, monthly, seasonal periodicities [4].

Chronoepidemiology [5] quantifies alterations of biologic rhythms with several frequencies as harbingers and possible determinants of the risk of developing certain diseases. Alteration of one or several rhythm characteristics in one or several hormones and other variables may reveal and quantify vulnerability or risk, prior to the occurrence of a given pre-disease or disease. This approach is positive (as compared to a negative ruling out of disease for the assessment of health), and personalized (instead of relying on the % morbidity or % mortality of a population).

Rhythmometric analysis can show significant parameters altered to determined times and it can give new information suggesting pre-symptomatic conditions.

Besides, chronobiological inquiry allows to point out sharp differences in a «risk variability», defining some characteristics (period, amplitude, phase). Periodicity also implies that a foreseeable event make sometimes take to prevention.

Circadian and ultradian «amplitudes» differ for circulating prolactin of clinically healthy women at

Table 1. – Amplitudes of least-squares spectral components of circulating prolactin in clinically healthy women in Kyushu, Japan (Jp) and Minnesota, USA (US)*

τ(h)	Jp	US	τ(h)	Jp	US	τ(h)	Jp	US
24.00	12.9 (*)	6.8 (*)	2.67	0.5	0.2	1.41	< 0.1	0.1
12.00	8.5 (*)	3.3 (*)	2.40	< 0.1	0.3	1.33	0.2	0.1
8.00	3.1 (*)	0.7	2.18	0.3	0.1	1.26	0.5	0.1
6.00	2.0 (*)	1.5 (*)	2.00	0.3	0.3	1.20	0.3	< 0.1
4.80	1.3	0.4	1.85	0.8	0.1	1.14	0.1	0.1
4.00	0.9	0.5	1.71	0.1	0.2	1.09	0.3	0.1
3.43	0.4	0.8	1.60	0.5	0.3	1.04	0.7 (*)	0.1
3.00	0.8	0.4	1.50	0.4	0.2	1.00	0.2	0.1

τ = trial period; JP = Japan; US = U.S. at low familial of developing breast cancer. Blood sampled every 20 min for 24 h in each season [13].
(*) $p < 0.05$ after correction for multiple testing for null hypothesis: amplitude at trial $\tau = 0$ [13].

low risk of developing breast cancer, as shown in Table 1. The harmonic trial periods are tested without any implication that some of them correspond to separate rhythms. The probably considerable extent to which these harmonics represent the circadian waveform is not here assessed.

At a given geographic site, spectral characteristics assess risk, and may reveal behaviour that differs when the variable investigated assumes values within *vs* outside the physiologic range. For instance, plasma aldosterone in clinically healthy women at a high risk of developing high blood pressure shows a lower rhythm-adjusted mean as compared to women at low risk, whereas, in the presence of overt pathology, adrenal tumors which secrete much aldosterone are associated with high rather than low blood pressure [6]. This inverse relation in health *vs* disease is also shown by children of parents with diagnosed high blood pressure who have a lower rather than a higher circulating aldosterone concentration as compared to that of children of parents with a negative diagnosis of high blood pressure [7].

The endocrine rhythms spectrum is altered long before an abnormality such as an elevation in blood pressure or some overt disease occurs: the circannual amplitude of aldosterone correlates negatively with the risk of developing high blood pressure.

The circannual amplitudes of prolactin and TSH correlate with the risks of developing breast cancer (in a longitudinal study [6]) and prostate cancer (in a transverse study [8]). Once the rhythmic structure has been mapped and related to wellness quantified by estimates of the innate and other risks of developing different diseases [9, 10], useful information may be cost-effectively gained from single or few time-specified determinations (e.g., on blood eventually drawn to integrate hormone concentrations over the span of one or a few ultradian cycles). A tentative constellation of endocrine classifiers for multiple disease risk assessment is shown in Table 2 [10].

The single samples underlying the computations leading to this table represent a snapshot on a roller coaster, were it not that the «pulsatile» or «episodic» ultradians are smoothed, if not integrated, by averaging accross individuals. In a given individual, the averaging of several samples over time or the drawing of a single sample slowly during an entire ultradian or even a circadian cycle is feasible. Some harbingers may also be found to constitute putative determinants of risk elevation as features of an early pathology. In certain seasons, a relatively low circadian Mesor of dehydro - epiandrosterone sulfate (DHEA-S) is a classifier and possibly a determinant of the risk of developing breast cancer [11] or of a personality prone to alcoholism [12]. If so, timed endocrine substitution treatment for lowering an elevated risk before there is overt disease will be indicated. This would constitute a major extension of hormone use from the classical endocrine substitution treatment in overt disease to the anticipatory time-targeted replacement of any relative deficiency for the prevention of widespread civilization disease, such as breast and prostate cancer, high blood pressure or a personality prone to alcoholism, if not to drug addiction [13].

An increased mortality after myocardial infarction may relate to both a reduced variability of normal heartbeats [14] and an amplified, phase-shifted circadian rhythm of abnormal (premature ventricular) beats in the 24 h ECG [15]. Two groups of patients were followed after a myocardial infarction. The premature ventricular beats in the 24 h ECG differed between those who were to suffer a sudden cardiac death within 5 years as compared to those who survived by considering the features of the circadian rhythm ($p = 0.002$), but not when only the average 24 h

Table 2. – Kinds and times of endocrine sampling recommended for further tests of multiple risk assessments

Season	Time-specified hormonal determination
	High blood pressure
Summer	TSH at 08:00 (IND) + Aldosterone at 00:00 (IND) or + Cortisol at 08:00 (IND)
Fall	Aldosterone at 00:00 or at 08:00 (IND)
Winter	Aldosterone at 00:00 (IND) + Prolactin at 00:00 (DIR) + DHEA-S at 08:00 (IND)
Spring	TSH at 00:00 (IND) + LH at 08:00 or TSH at 08:00 (IND) + Aldosterone at 09:40 (DIR) or + E1 at 08:00
	Breast cancer
Summer	Insulin at 00:00 (IND) or E1 at 00:00 (IND) or E1 (IND) + TSH (DIR) + Aldosterone (IND), all at 08:00
Fall	Prolactin at 00:00 (IND) or Prolactin + E2 + T3 (IND) all at 08:00
Winter	17-OH progesterone at 00:00 or Prolactin (IND) + Cortisol (DIR) + T4 (IND) + 17-OH progesterone, all at 08:00
Spring	Insulin at 18:40 (IND) or T4 at 09:40 (IND) or LH at 09:40
	Expansive personality (possibly related to alcoholism and drug abuse)
Summer	DHEA-S at 12:00 (IND)
Fall	DHEA-S at 00:00 (IND) + Prolactin at 00:00 + LH at 08:00 (IND)
Winter	DHEA-S at 08:00 (IND) + Prolactin at 00:00 + LH at 08:00 (IND)
Spring	Cortisol at 18:40 (DIR) + TSH at 18:40 (IND)

DIR = direct relationship; IND = indirect relationship [13]

incidence of premature ventricular beats was used ($p = 0.084$). Variability without or preferably with chronobiometry adds another dimension to the analyses of pathologic features such as premature ventricular beats.

Novel information not obtained by conventional location or dispersion indices can indeed be provided by the computation of the circadian and other amplitudes beyond sudden death following myocardial infarction. The importance of chronobiologic endpoints has been demonstrated in several cases of cardiologic and broader epidemiologic interest. Rhythm characteristics serve on a group basis as classifiers and potential harbingers not only of sudden cardiac death, but also as earliest risk indices applicable by the time of birth [16]. The amplitudes of several rhythmic components with periods of 1.5, 3, 12 and 24 h and of 7 days and 1 year, of systolic or diastolic blood pressure separate groups of human newborns with a positive *vs* negative family history of high blood pressure and/or cardiovascular diseases when the mean based on the same data does not do so. These amplitudes also undergo secular changes, yet such changes notwithstanding, on a group basis, indicate the effects of in utero exposure to betamimetics [17].

Chrono-risk: a determinant in preventive medicine

Chronobiological approach can show another potential main interrelation among several risk factors: the so called *chrono-risk*.

As are many diseases with competing risks, atherosclerosis and correlated events seems to be a process occurring in several stages involving various risks factors, some near and some distant at the event.

Classical epidemiology includes, with a family history, cholesterol, diabetes, blood hypertension, diet, fibrinogen, personality, smoke, etc. These factors vary in their reliability as predictor of clinical events of atherosclerosis (CE). Moreover, the effects of established risk factors are complicated by the likely coexistence of as yet unknown protective and/or harmful factors [18].

Against this background, it is not surprising that the definition of high-risk groups using the conventional risk factors singly and in combination has been described as unsatisfactory and disappointing. New ways of identifying high-risk subjects are needed for a sharpening of the risk gradients. Among the factors suspected of playing a role in the development of CE, one must recognize the fact that several *metabolic* variables and functions are

usually rhythmic with several frequencies - in conjunction with trends associated with growth, development and aging. This circumstance complicates the interpretation of results from a single time-unspecified test for the purpose of allocating an individual's risk status. In this context, a chronobiologic approach is mandatory: it is cost-effective if it avoids pitfalls by suggesting time-dependent kinds of laboratory tests; it is useful, if it reveals new relationship among risk factors, notably time-related ones.

Some potentially harmful factors, in fact, could reveal rather similar timing: furthermore, the acrophases of these risk variables, on the one hand, and those of potentially protective factors, on the other hand, may be in near antiphase.

Traditionally, the medical risk is assessed by controlling biological variables (risk factors) in their normality against the pertaining reference limit.

The «chrono-risk» emphasizes the fact that the medical risk may be given by the temporal phase of endogenous biological rhythms in subjects with currently accepted low or zero risk load.

The concept that the risk for human health may be in abnormal timing of biological functions should cause medicine to abandon the attitude to consider the quantitative deviations the exclusive source of risk.

Almost ten years ago [19], we pointed out that atherosclerotic risk load could change in accordance with convergencies or not of protective and/or dangerous factors in some times of circadian stage (circadian chrono-risk). More recent studies [20] in the same field have shown that clinical events of ischemic heart disease generally prefer the morning hours: there is a remarkable similarity among the circadian patterns of onset of fatal and nonfatal myocardial infarction [21], sudden cardiac death [22], thrombotic stroke [23], and transient myocardial ischemia [24] (Fig. 2).

These studies consider that periodicity is due to a different incidence of risk factors. There are extensive data supporting that well known risk factors (aggressive or protective) for ischemic heart disease and its acute events, vary periodically. The features (phase, amplitude and level) of metabolic, endocrine system, blood pressure, coronary tone, fibrinolysis, some components of clotting process, etc. are not random distributed (Fig. 3) in the different periods (circadian, ultradian and infradian) but they form a network, a system measurable in rhythmometric terms, and partly conditioning an equilibrium, that is the individual health condition.

Several researches suggest that platelet function is also an important factor associated with the onset of acute thrombosis and may contribute to the progression of coronary atherosclerosis and to myocardial infarction [25, 26]. It is well-documented that platelets play a part in the thrombotic process, and it is likely that the degree of platelet aggregability at the time of plaque rupture influences whether complete vascular occlusion will ultimately occur. Recent evidence suggests that spontaneous platelet aggregation, independent of other cardiovascular risk factors, may be an important factor in predicting the occurrence of acute vascular occlusion [27].

The opportunity to assess directly whether aspirin alters the circadian variation of acute thrombosis *in vivo* may be provided by the U.S. Physicians' Health Study, a randomized, double blind, placebo-controlled trial of low-dose aspirin among 22,071 apparently healthy male physicians [28, 29]. The effect of low-dose aspirin in preventing acute infarction was not equally distributed [30] over the 24 h of the day. Rather, aspirin appeared to blunt the overall circadian variation of acute infarction (Fig. 4). Specifically, aspirin was associated with a 60% reduction in the incidence of infarction during the morning waking hours, compared with a 34% reduction for the remaining hours of the day.

While aspirin reduced the morning peak of infarction in this study group, it is also clear that the aspirin did not completely abolish the circadian

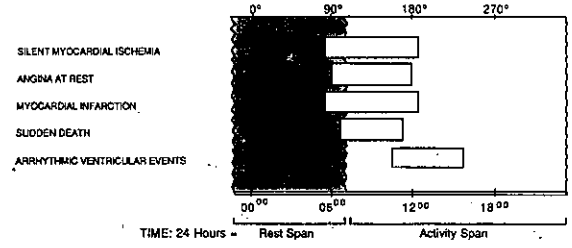

Fig. 2. - Clinical manifestations of ischemic heart disease. Achrophasogram (constructed from several Authors) of daily distribution in the frequency of several cardiovascular events.

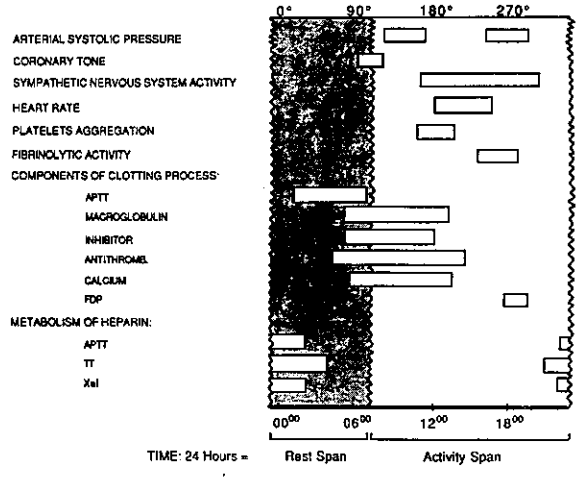

Fig. 3. - Parameters probably related to clinical events of ischemic heart disease. Achrophasogram (constructed from several Authors) of daily distribution of several clinical-laboratory parameters potentially correlated with events of ischemic heart disease.

Table 3. – Circadian response rhythm of TxB2 to IB in clinically healthy subiects (*)

	p	MESOR ± SE	Amplitude (95% CI)	Acrophase (95% CI)
Reference	0.384	361 ± 37	40.5	– 68°
Washout	0.058	373 ± 27	66.7	– 206°
Rx at 08:00 (day 1)	0.017	57 ± 8	30.8 (13.1; 48.5)	– 66° (– 48; – 87)
(day 14)	< 0.001	71 ± 10	62.3 (38.4; 86.2)	– 55° (– 45; – 69)
Rx at 20:00 (day 1)	0.001	34 ± 5	24.9 (17.0; 32.8)	– 280° (– 257; – 313)
(day 14)	< 0.001	53 ± 11	47.9 (26.8; 68.6)	– 251° (– 239; – 276)

* CI = Confidence Interval; Acrophase in (negative) degrees, with 360° = 24 h; 0° = 00:00.

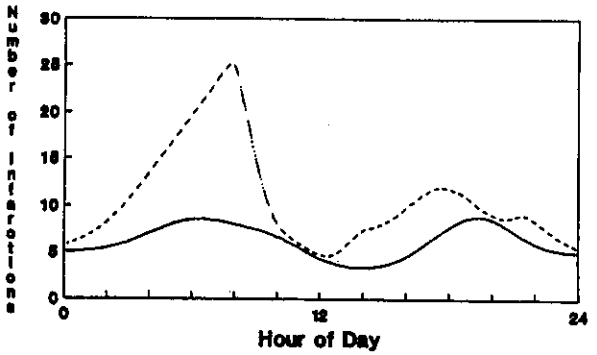

Fig. 4. - The hourly frequency of myocardial infarction onset in a randomized, placebo-controlled trial showing the effect of aspirin on blunting the morning peak of acute thrombosis; ——— = aspirin; — — = placebo. [30].

pattern of infarction onset. This finding is in agreement with the hypothesis that several factors in addition to platelet aggregation determine acute onset of disease.

In this intricate multi-component and multi-frequency system the variation of risk load is not only due to the variation of risk factors levels, but to the phase relationship, to amplitude and to the gradient resulting at a fixed time (*body hour*) in every single subject.

Drugs or other procedures able to reduce the level of a risk factor and to shift their phase, could be useless, or dangerous: further they could produce striking effects, for a small level or phase variation. The daily administration of Indobufene (IB), a potent inhibitor of platelet aggregation, apart from decreasing thromboxane B2 (TxB2) ($p < 0.001$), induces a circadian response pattern with an acrophase that depends on the timing of IB administration and an amplitude that increases from the first to the last day on the treatment ($p < 0.10$), (Table 3).

In this contesct also drugs time administration contributes to risk variation. Thus every change induced in the multitude of variabilities implied in chronorisk, produces effects which are the result of factors differing in time.

The same risk load can be obtained through completely different ways; so that the risk evaluation must be individual and also therapeutic approach has to be different and individual in the clinical aspects of CE.

Possible indentification of changes in these variabilities, should perhaps allow an early prediction and, may be, prevention of pre-symptomatic conditions. The demonstration that weekly cycles in some components of lipidic system [31], a circadian, circaseptan [32], ultradian and circannual periodicity [33] in CE, imply multiple intricate interrelations even among social, seasonal events and biological timing structures: at present physicians still perceive all these middled elements as «biological noise».

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