



The Biochemical Fundamental Biomarkers of The Status of Health Against Cancer and Cardiovascular Diseases: Presence of Cortisol and Melatonin Circadian Rhythms, Normal Blood Levels of Fatty Acid Amide Hydrolase (FAAH) and Transforming Growth Factor-Beta (TGF-Beta), and Normal Values of Lymphocyte-to-Monocyte Ratio (LMR) and Atrial Natriuretic Peptide (ANP)-to-Endothelin-1 (Et-1) Ratio

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ABSTRACT

The future of laboratory analyses would require the identification of clinical parameters involving the main integrative biological functions including neuroendocrine, immune and cardiovascular systems, and capable of predicting the evidence of metabolic alterations and the possible occurrence of systemic diseases. According to the clinical data available up to now, the status of health may be identified by the following five major biomarkers, consisting of normal circadian rhythm of cortisol and the pineal hormone melatonin, normal blood concentrations of fatty acid amide hydrolase (FAAH), whose increase is associated with an endocannabinoid system deficiency, normal lymphocyte-to-monocyte ratio (LMR) values, normal blood levels of TGF-beta, the main immunosuppressive anti-inflammatory endogenous molecule, and normal values of ANP-to-ET-1 ratio. Since the evidence of alterations involving these five parameters may predispose to the onset of more severe metabolic disorders or systemic disease, the clinical evaluation of these five biomarkers could constitute the routinary laboratory analyses for realizing a real Preventive Medicine.

Introduction

Most laboratory analyses are simply performed to demonstrate possible already present metabolic alterations, such as the evidence of abnormally high values of glucose or cholesterol to establish the diagnosis of diabetes or hypercholesterolemia. Then, it would be important from a clinical point of view to identify new laboratory parameters, whose alterations may precede, predict, and determine the possible occurrence of more severe disturbances, including metabolic, neoplastic, psychiatric and cardiovascular diseases. According to the data available up to now in the literature, within the great number of possible biomarkers, whose alteration already in the absence of evident subjective clinical symptoms may allow more severe biological alterations, the main and most simple to be measured laboratory parameters, which synthetically reflect the status of health of the whole living organism, may be represented by five fundamental biomarkers, consisting of: 1) the evidence of a physiological circadian rhythm of cortisol¹ and the pineal

indole hormone melatonin (MLT);² 2) normal values of lymphocyte-to-monocyte ratio (LMR); 3) normal blood concentrations of TGF-beta⁴; 4) normal blood levels of fatty acid amide hydrolase (FAAH), the enzyme which destroys the endogenous cannabinoids⁵; 5) normal atrial natriuretic peptide (ANP)-to-endothelin-1 (ET-1) ratio^{6,7}. Until few years ago the main integrative biological systems represented by the neuroendocrine, the immune and the cardiovascular systems, were separately investigated, and they were substantially studied only in relation to their specific activities, the endocrine secretions for the endocrine system, the psycho-neuro-biological effects actions for the nervous system, and the immune responses for the immune system with its cytokine network, which may allow the immune cells to act on the other immune cells and biological systems without the necessity of a cell-cell contact. On the contrary, as well as demonstrated by the recent advances in the area of the Psycho-neuro-endocrino-immunology (PNEI), endocrine, nervous, and immune systems are connected by reciprocal and complex interactions. In fact, it has been shown that immune cells may express receptors for hormones, neurotransmitters and neuromodulators, and they are sensitive to their actions. On the same way, endocrine and nervous cells may express cytokine receptors, and be influenced by their effects. Finally, more recently it has been demonstrated that heart itself may exert both immunostimulating and immunosuppressive effects through its endocrine-like activity, namely consisting of the production of atrial natriuretic peptide (ANP)⁶, and endothelin-1 (ET-1)⁷. ANP has appeared to exert immunostimulatory and anti-inflammatory activity⁶, whereas ET-1 may play an immunosuppressive pro-inflammatory action⁷. However, despite the complexity of the biological connections, in addition to its fundamental role for hemodynamic reasons, the heart would also exert a dominant role in the neuromodulation of the immune responses^{6,7}. ANP is namely produced by left atrial myocytes, while ET-1 is also produced by the endothelial cells. In more detail, the link between cardiovascular disorders and immune alteration-related systemic diseases, including cancer and autoimmunity, would be mainly realized by the balance between ANP and ET-1 blood concentrations, because of the multiple biological activities of both ANP and ET-1, including cardiovascular, metabolic, inflammatory and immunomodulating effects. In particular, it has been shown that ET-1 may represent one of the main endogenous factors responsible for both cardiovascular pathologies, including essential hypertension, myocardial ischemia, heart failure, pulmonary hypertension and atherosclerosis^{7,8}, and neoplastic diseases, because of its inflammatory, immunosuppressive, angiogenic⁹ and tumor growth factor activities¹⁰. On the contrary, ANP has been proven to protect against both cardiovascular

pathologies⁶, including arterial hypertension, heart failure, and myocardial ischemic and arrhythmic diseases, and neoplastic diseases because of its direct antiproliferative antitumor activity¹⁰, anti-angiogenic action, anti-inflammatory and immunostimulatory effects, mainly due to the activation of T lymphocyte functions¹¹. Then, from an imagination point of view, these five biomarkers could be considered as a laboratory Torah of the status of health, since they could put into evidence preliminary anomalies involving the biological response, which is substantially a psycho-neuroendocrine-angio-immune response, with respect to most other common laboratory analyses, which simply document the great variety of possible alterations following those involving the afore mentioned five fundamental parameters of the status of health. Then, the clinical question is to justify their importance in the modulation of the biological response on the basis of well defined pathogenetic mechanisms in relation to each single biomarker. By considering the fundamental importance of the unity between the single living organism and the universal light and energy conditions, it is obvious that the primary biomarker of the status of health would have to be represented by the presence or the absence of a physiological circadian rhythmicity in the biological functions, which is synthesized by the investigation of cortisol and MLT circadian rhythms.

The Clinical Importance Of Cortisol And Melatonin Circadian Rhythms

In the healthy subjects, the human body is physiologically able to change itself in relation to the different light and environmental conditions during the day, as well as in relation to the different seasons. The capacity of living organisms of modifying themselves in relation to the universal conditions is defined as synchronization status¹², which is namely expressed by the evidence of a physiological circadian variations in the daily secretions of both cortisol, with high values during the morning and low levels during the afternoon¹, and the pineal hormone MLT, whose concentrations are higher in the night, with a following existence of a well defined light/dark MLT rhythm². The evidence of the lack of a circadian rhythm of cortisol and MLT secretions is the expression of desynchronization process, and it has been proven to predict a poor prognosis in metastatic cancer patients^{13,14}, while its significance in autoimmune diseases is still controversial. Alterations of cortisol and MLT rhythms have been described also in depression¹⁵, with increased cortisol production and diminished nocturnal secretion of MLT. In any case, it has to be taken into considerations that aging is associated with a progressive decline in the diurnal variations of both cortisol and MLT levels, and aging itself is defined as a progressive failure and lack of the biological circadian rhythms, including that of the blood pressure^{1,2}.

Even though few data only are available, the alterations of cortisol circadian secretion tend to be associated with concomitant alterations of MLT light/dark rhythm¹⁶, by probably reflecting a similar functional neurochemical damage involving the suprachiasmatic nucleus. MLT circadian rhythm may be evaluated into the blood, or by measuring the day and night urinary excretion of the main metabolite of MLT, the 6-sulphatoxy-melatonin (6-MTS). In any case, it would be remarked that from a clinical point of view, the investigation of MLT rhythms more important than that of cortisol, since a decline in the nocturnal production, with a consequent progressive lack of its normal light/dark rhythm, has been seen in both brain and cardiac ischemia^{17,18}, and it has appeared to be associated with a worse prognosis because of the neuroprotective and cardioprotective activity of MLT itself^{19,20}. In addition, the pineal deficiency occurring during the clinical course of the neoplastic diseases, with a consequent decline in MLT levels namely during the night, would constitute the main cancer-related endocrine failure, which predicts a worse prognosis, because of the fundamental anticancer role of MLT, due to both antiproliferative cytotoxic and immunostimulatory effects on the antitumor immunity^{21,22}, namely due to the stimulation of the secretion of the two main antitumor cytokines in humans, consisting of IL-2 and IL-12^{21,22}.

The Clinical Importance Of Fatty Acid Amide Hydrolase (Faah) For Investigating The Endocannabinoid System

At present, it is known that the endogenous brain and body cannabinoid system plays a fundamental role in the control of several metabolic, nervous, endocrine and immune functions, as well as in the modulation of the psychospiritual life by exerting an essential function in the control of pain, perception of pleasure and expansion of mind²³. The two main endocannabinoid agents, both synthesized from the arachidonic acid, are represented by arachidonyl-ethanol-amide (AEA), also-called anandamide because of its psychedelic properties, and 2-arachidonyl-glycerol (2-AG)²⁴. Both AEA and 2-AG shows a circadian rhythm in their secretion, with highest levels of AEA during the night and highest values of 2-AG during the light phase of the day²⁴. Both endogenous and exogenous cannabinoid agents are metabolized and destroyed by the enzyme FAAH^{23,24}. Then, the evidence of abnormally high blood levels of FAAH has been proven to be associated with low levels of endocannabinoids, because of FAAH- induced cannabinoid degradation, with a following deficiency in the functionless of the endogenous cannabinoid system, the so-called endocannabinoid deficiency²⁵. Obviously, because of its fundamental role in the modulation of the psychobiological life, an endogenous cannabinoid deficiency may allow important metabolic and psychic

disorders. The evidence of abnormally high blood levels of FAAH, with a consequent endocannabinoid deficiency, has appeared to be associated with a worse prognosis in most systemic human inflammatory diseases, including cancer and cardiovascular diseases²⁶, and this finding is not surprising by taking into consideration the fundamental role of the cannabinoid agents in the modulation of the immuno-inflammatory biological response by regulating the cytokine network²³⁻²⁶. In particular, it has been shown that the activation of the endocannabinoid system may stimulate the anticancer immunity^{23,24} through its connection with the pineal gland²⁷, which plays an essential role in the natural immunobiological resistance against cancer onset and development²⁸ through several and complex mechanisms, including a modulatory effect on the cytokine network with stimulation of IL-2 and IL-12 release, and inhibition of macrophage-related cytokines, such as IL-6 and TNF-alpha²⁹, an anti-angiogenic activity, and a direct cytostatic cytotoxic antitumor activity³⁰. Therefore, FAAH blood levels may be considered as a synthetic biomarker of the whole function of the endocannabinoid system. Obviously, the main common target for cortisol, pineal hormone MLT and endocannabinoid agents is represented by the same immune system, then the cytokine network, whose functional status according to recent observations³ may be clinically investigated through the simple evaluation of LMR.

The Clinical Importance Of Lymphocyte / Monocyte Ratio For Evaluating The Immune Status

The functionless of the whole immune system would substantially depend on the equilibrium between immunostimulatory and immunosuppressive events. The inflammatory response may amplify some immune responses, such as those against pathogenic agents, but suppress other immune response, in particular, the antitumor immunity³¹. The suppression of the immune responses, including the antitumoral immunity, is mainly mediated by the macrophage system through the release of several pro-inflammatory immunosuppressive cytokines, namely IL-1 beta, IL-6 and TNF-alpha³¹ and by regulatory T (T reg) lymphocytes, which inhibit the immune responses namely through the release of TGF-beta³², whose generation, however, would be promoted by monocyte-macrophage system itself. In fact, it has been demonstrated the decline in LMR, due to both lymphocyte decrease and monocyte increase, is associated with a concomitant increase in T reg cell number and activity³³. Moreover, the degree of the activation of the macrophage system has been shown to be positively correlated to monocyte count³¹, while lymphocyte number would mainly reflect TH1 cell count³⁴. Therefore, the simple LMR would constitute a synthetic clinical biomarker, which reflects the whole immune functionless, in particular, the balance

between lymphocyte and monocyte-macrophage systems. In fact, it has been shown in metastatic cancer patients that the evidence of a progressive decline in LMR is associated with a poor prognosis in terms of both survival time and response to the various anticancer therapies, including chemotherapy and immunotherapy^{33,35}, by reflecting an enhanced T reg cell activation. LMR would play a prognostic significance also in the cardiovascular diseases, since the occurrence of abnormally low values of LMR may predict a worse prognosis in patients with myocardial infarction³⁶, because of the fundamental role of T lymphocytes in tissue repairing and the amplification of ischemia-induced tissue damage due to the inflammatory response induced by the macrophage system.

The Clinical Importance Of Tgf-Beta Blood Concentrations

Today, it is known that there are two different potential origins of the inflammatory response, which may be induced by the monocyte-macrophage system or by the TH-17 lymphocytes (TH17)³⁷, which are linked by reciprocal stimulatory interactions. On the other hand, the inflammatory response is mainly inhibited by T reg cells through the release of TGF-beta. However, since TGF-beta may be produced by both T reg lymphocytes and monocyte-macrophage cells, which also mediate the inflammatory response, the clinical inflammation-related symptoms of patients do not allow to suppose which may be the blood levels of TGF-beta. Then, the determination TGF-beta blood levels would represent a fundamental biomarker, which can be substituted by no other laboratory parameter and measured in an attempt to put into evidence two possible different inflammatory conditions characterized by low or normal-high values, since no other immune parameter, including LMR values themselves, may predict TGF-beta blood concentrations. In fact, if we consider that blood levels of inflammatory cytokines, such as IL-1-beta, IL-6 and TNF-alpha, are substantially related to monocyte count, as well as that those of IL-2 and IL-12 are namely in relation to lymphocyte count, TGF-beta is the only cytokine, whose blood concentrations cannot be predicted on the basis of the simple LMR. In autoimmune diseases, the evidence of abnormally low levels of TGF-beta has been shown to be associated with a more severe inflammatory status and a worse clinical prognosis³⁸, whereas the presence of normal or high TGF-beta levels is associated with a clinical remission of disease. Similar results have been reported in the cardiovascular diseases³⁹. On the contrary, the evidence of abnormally high blood levels of TGF-beta has been shown to predict a poor prognosis in most advanced cancer patients⁴⁰, because of its immunosuppressive activity on the anticancer immunity. Finally, the complex neuroimmune interactions are under a central modulatory control realized by the heart, in an attempt to regulate

the immunological response in relation to the general condition of the whole organism.

The Clinical Importance Of Anp-To-Et-1 Ratio For Evaluating The Cardiovascular Function

Despite its complexity, the status of health of the cardiovascular system, as well as of its neuroendocrine regulation would be synthetically maintained by a perfect equilibrium between ANP and ET-1 secretions, since ANP may counteract the overall negative biological effects of ET-1 on both cardiovascular and immune systems⁷⁻¹⁰. However, since the status of health is not related to their blood concentrations, but also on their ratio, the measurement of ANP-to-ET-1 would constitute a more appropriate clinical parameter with respect the single detection of ANP or ET-1, as demonstrated in ischemic diseases⁴¹. The physiopathological significance of ANP/ET-1 ratio and its prognostic importance is justified by the fact that it does not reflect the simple endocrine cardiac activity, but the whole neuroendocrine control of both cardiac and vascular functions, particularly because the connections among ET-1, renin-angiotensin system and antidiuretic hormone (ADH) secretion⁴², as well as among ANP, oxytocin and the pineal gland^{43,44}, which are connected by reciprocal stimulatory effects.

Conclusions

The elaboration of a real Preventive Medicine would require the availability of laboratory parameters provided by an olistic value and capable of preceding the occurrence of more severe metabolic or degenerative pathologies. The afore mentioned five clinical biomarkers could constitute a first clinical example in the future evolution of the medical Sciences.

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