**Introduction**

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders of the stem cell, often evolving in acute leukemia. They are characterized by anemia, and it has been attributed either to a deficiency in erythropoietin (EPO) secretion or to a resistance to EPO itself. Since in healthy subjects the serum circulating EPO levels fluctuate during the day, the aim of the study was to investigate the diurnal rhythm of EPO in MDS. Two groups of subjects were admitted to the study: (A) 20 adult clinically healthy subjects, and (B) 20 patients with MDS without renal failure. After standard life conditions in hospital lasting one week, venous blood samples were drawn during the span of a whole day and every four hours, starting from midnight, for the measurement of serum EPO levels by RIA. Statistical analysis was carried out by means of the "cosinor" method. The results show that the controls present a significant ($p < 0.05$) circadian rhythms in serum EPO levels with acrophase in the late afternoon; on the contrary, no significant ($p > 0.05$) rhythm was detected in patients with MDS. Patients with MDS presented significantly higher ($p < 0.05$) MESOR and lower ($p < 0.001$) amplitude of EPO circadian rhythm in respect to the controls; moreover, a significant ($p < 0.005$) difference was found between the two groups in overall EPO rhythm. These data confirm the existence of a physiological circadian rhythm in serum EPO concentrations with maximum in the afternoon. Because EPO levels are increased in the patient group, EPO deficiency does not seem to be the cause of anemia in MDS. Reduced EPO amplitude may be a compensatory mechanism for enhancing its activity in MDS. Finally, the stimulatory therapy in MDS with recombinant human EPO should be administered in the late afternoon hours, in order to respecting and simulating the physiological circadian rhythm of endogenous EPO.

**Key Words:**
Circadian rhythm, Erythropoietin, Myelodysplastic syndromes.

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**Abstract.** – Myelodysplastic syndromes (MDS) are a group of clonal disorders of the hemopoietic stem cell, often evolving in acute leukemia. They are characterized by anemia, and it has been attributed either to a deficiency in erythropoietin (EPO) secretion or to a resistance to EPO itself. Since in healthy subjects the serum circulating EPO levels fluctuate during the day, the aim of the study was to investigate the diurnal rhythm of EPO in MDS. Two groups of subjects were admitted to the study: (A) 20 adult clinically healthy subjects, and (B) 20 patients with MDS without renal failure. After standard life conditions in hospital lasting one week, venous blood samples were drawn during the span of a whole day and every four hours, starting from midnight, for the measurement of serum EPO levels by RIA. Statistical analysis was carried out by means of the "cosinor" method. The results show that the controls present a significant ($p < 0.05$) circadian rhythms in serum EPO levels with acrophase in the late afternoon; on the contrary, no significant ($p > 0.05$) rhythm was detected in patients with MDS. Patients with MDS presented significantly higher ($p < 0.05$) MESOR and lower ($p < 0.001$) amplitude of EPO circadian rhythm in respect to the controls; moreover, a significant ($p < 0.005$) difference was found between the two groups in overall EPO rhythm. These data confirm the existence of a physiological circadian rhythm in serum EPO concentrations with maximum in the afternoon. Because EPO levels are increased in the patient group, EPO deficiency does not seem to be the cause of anemia in MDS. Reduced EPO amplitude may be a compensatory mechanism for enhancing its activity in MDS. Finally, the stimulatory therapy in MDS with recombinant human EPO should be administered in the late afternoon hours, in order to respecting and simulating the physiological circadian rhythm of endogenous EPO.

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Circadian rhythm, Erythropoietin, Myelodysplastic syndromes.
etic megaloblastoid features with ineffective erythropoiesis\textsuperscript{[1,2,4,5]}. On the basis of these data, it has been hypothesized that the primary abnormality of the hemopoietic cells and their failure to grow adequately are due to a failure of response to cytokines\textsuperscript{6}, or that anemia is due directly to a deficiency in the renal EPO production\textsuperscript{7}.

EPO is a glycoprotein produced mainly by the peritubular cells of the kidney and also by the liver. It has a predominant effect on the committed erythroid cells, colony-forming unit-erythroid, promoting their proliferation and differentiation into proerythroblasts. EPO may also stimulate the differentiation of a more primitive erythroid progenitor, the burst-forming unit-erythroid, in association with so-called burst-promoting activity; EPO also interacts with other hematopoietic growth factors to promote the production of megakaryocytes\textsuperscript{8,9}. EPO is detectable in the serum\textsuperscript{10} and shows large fluctuations during the 24-hs period, with a well-marked circadian rhythm\textsuperscript{11,12,13} with maximum levels in the afternoon, not influenced by the aging process\textsuperscript{14}.

Therefore, the aim of the study was to investigate the circadian rhythms of the circulating serum levels of EPO in MDS, in order to verify whether these hematological disorders are associated with quantitative or qualitative changes in its circadian rhythm.

**Materials and Methods**

Two group of subjects were admitted to the study: Group A: 20 adult clinically healthy normocyticemic subjects, 13 males and 7 females, aged 55-75 years (mean age = 65.3 ± 6.4 yrs), with mean serum hemoglobin = 13.5 ± 1.7 gr/dl, and mean serum creatinine = 1.23 ± 0.14 mg/dl; Group B: 20 patients with MDS, 14 males and 6 females, aged 62-79 years, (mean age = 73.2 ± 3.8), with mean hemoglobin = 8.2 ± 1.3 gr/dl, and mean serum creatinine = 1.15 ± 0.21. All patients met the FAB diagnostic criteria for MDS\textsuperscript{1}: there were 6 cases of RA, 4 of RARS, 5 of RAEB, 3 of RAEB-t, and 2 of CMML. The patients had not received drugs and/or blood transfusion in the month preceding the study.

After standard life conditions in hospital lasting one week, with sleep and/or rest period from 22:00 to 6:00, and meals at 8:00, 12:30 and 18:30 with free diet, venous blood samples were drawn from a peripheral vein of each subject during the span of a whole day and every four hours, starting from midnight. The serum levels of EPO were assayed in each sample by means of a commercial radioimmununassay\textsuperscript{15}, with precipitation by the double antibody technique; the serum samples were assayed as a batch in two independent assays, and the EPO concentrations were recorded as the geometric mean values of these two estimates; the mean interassay coefficient of variation of estimates was 7.0%. The normal range using this technique for serum EPO concentration is 5-100 U/L.

The time related values of EPO were subjected to statistical analysis using chronograms (mean ± 1SD) and to inferential circadian statistical analysis by means of the "mean-group cosinor" method\textsuperscript{15}. This method is able to detect a significant (\(p < 0.05\)) circadian rhythm and the rhythm parameters: MESOR (average level of rhythm), amplitude (length from MESOR to acrophase), and acrophase (peak of rhythm). The circadian rhythms of EPO were compared among the two groups by means of the "Hotelling's statistic test"\textsuperscript{15}.

**Results**

The serum levels of EPO fluctuated during the day in each group. When the data were analyzed by "cosinor" method, the controls (group A) presented a significant (\(p < 0.01\)) circadian rhythm for the circulating serum EPO levels, with the acrophase in the late afternoon and the lowest values during nighttime; no rhythm (\(p > 0.05\)) was detected in MDS patients (group B). The MDS patients had significant (\(p < 0.01\)) higher MESOR and significant (\(p < 0.001\)) lower amplitude of EPO than the controls; significant difference (\(p < 0.005\)) was demonstrated between the overall rhythms of the two studied groups.

Figure 1 illustrates in detail the results, both as chronograms and as derived by "cosinor" analysis.
**Discussion**

This study extends to MDS our previous investigations on the circadian rhythm of EPO in course of multiple myeloma. Since the EPO circadian rhythm was preserved in patients without renal failure and lost in those with renal failure, we have hypothesized that the renal impairment in course of myeloma, and not the disease itself, is the main cause of anemia and of the loss of the EPO circadian rhythm in these patients.

The present observations confirm that serum EPO presents in healthy subjects a definite circadian rhythm, with peak in the afternoon. The mechanisms behind the observed circadian rhythm are still unclear. The accepted stimulus for EPO production is the tissue hypoxia, but this does not explain the rise in the afternoon hours of the hematopoietic growth factor. It is hypothesized that, since several of the hormones secreted or regulated by the pituitary gland show circadian rhythm, their rhythms may be involved in the control of the EPO circadian rhythm; moreover, changes in release rate and in metabolism of EPO and variations of the blood flow through the kidney during the day have to be considered.

The diurnal rhythm of EPO is deranged in patients with MDS, suggesting, together with the observation of a higher MESOR, that EPO deficiency does not seem to have a fundamental role in the pathogenesis of anemia in the patients with MDS. Endogenous serum EPO levels in MDS are extremely variable;
approximately one-third of patients have levels that are inappropriately low for the degree of anemia, and an inverse relationship between the level of EPO and the degree of anemia has been found. Moreover, no correlation has been demonstrated between serum EPO concentration and total erythroid production. On the other hand, the lower amplitude supports the hypothesis of a resistance to the activity of the growth factor. In fact, the reduced amplitude, with constant daily serum levels, represents, from a chronobiological point of view, a compensatory mechanism: constant levels of any biological active substance have the same effectiveness of higher, but fluctuating, levels.

A circadian difference exists in the susceptibility of the marrow to the effect of radiation, myelotoxic drugs and growth factor, and in vivo EPO administration enhances daily rhythms in erythroid colony numbers by increasing their amplitudes while leaving their circadian shapes virtually unchanged. The observation that the increment in erythroid colony numbers after EPO administration varies up to 16-fold with the time of day of treatment, and the present demonstrations that EPO presents its higher values in the afternoon and that this rhythm is lost in patients with MDS suggest that the therapy with recombinant human EPO, that actually represent a supportive therapy for the MDS patients, with a response in the 20-30% of cases, should be administered in this time of the day. This time of administration, in fact, should resemble its own physiological circadian rhythm, simulating and respecting the endogenous hemopoietic growth factor.

In effect a study on patients with renal failure undergone to hemodialysis has demonstrated that the evening intravenous administration of recombinant human EPO seems to have a quicker therapeutic success.

References


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