FLUCTUATIONS OF CD34+ CELLS NUMBER IN BLOOD OF CANCER PATIENTS DURING FINAL YEAR OF LIFE

The purpose of the work was the comparative study of dynamic relations between the fluctuation of survival rate and presence of hematopoietic progenitor cells in circulating blood of oncologic patients in the final months of their life. The registered associativity turbulence in the content of CD34+ cells in a blood of the advanced oncologic patients and turbulence of probability of their death leads to the assumption that the reason for life’s cessation may be the abnormal morphogenic activity of young cells produced in the bone marrow toward the tumor tissues of the host, as well as a lack of such activity toward the critical normal tissues. Thus, the cytotoxic, especially long-term, therapy of patients with advanced cancer may be timely even harmful for them and compromise the ultimate result of treatment.

The regimes of cytotoxic therapy of patients in cases of premature exhaustion of hematopoietic resource should be optimized based on individual periodicity of blood saturation with progenitor cells. The development of reliable and simple methods for clinical monitoring of quickly changing prognosis of patients status seems to become important aim of coming investigation. The data might be useful for optimization of regimes of cytotoxic therapy of individual patients in emergency.

**Keywords:** Cancer, survival, life span, monthly risk of a mortality, periodicity, blood stem cells, approximation

**UDC:** 616-006-036.88

**Introduction**

The weakening of hematopoietic function in carrying out conventional cytotoxic therapy of solid forms of malignancy is well-known (Cancer Therapy Evaluation Program, 1999). It is officially qualified as the almost inevitable consequence of the basic treatment (U.S. Department of Health and Human Services, 2006). Unlike systemic oncological diseases where necessity of inhibition proliferative activity of a hematopoietic tissue is pathogenetically expedient, in solid forms of malignancy the hematodepression, accompanying treatment, looks contradicting conceptually to popular representation about an antitumor function of the immune system. The system of lymphocytopoiesis is a most sensitive part of the general hematopoiesis to damaging action of cytotoxic chemotherapy and radiotherapy. Moreover, in the light of the newest data about the participation of T-lymphocytes in formation of vessels and about the ability of hematopoietic stem cells to support of cell's renewing in tissues of a various origin, including tumors (Drapeau, 2010; Wright, Wagers, Glati, Johnson, and Weissman, 2001; Weidt, Niggemann, Kasenda, Drell et al., 2007; Dalakas, Newsome, Harrison, and Plevris, 2005; Kucia, Ratajczak, and Ratajczak, 2005; Mineo, Ambrogi, Baldi, Rabitti et al., 2004; Trempus, Morris, Ehinger, Elmore, Bortner et al., 2007; Hur, Yang, Yoon, Lee, Park et al., 2007; Ho, Wagner, and Mahlknecht, 2005), the therapeutic hematodepression does not look like as just side effect or annoying “complication”, and needs to be investigated comprehensively. The theoretical discussion arguments in favor of necessity of recognition of hematological “complications” as signs of effective treatment (Propert and Andersen, 1988) were put forward earlier but did not get its continuation. Data on 10-20 times variation of personal stock of so-called primitive precursors cells including pluripotent stem cells, endothelial precursors and hemangioblasts in a blood of healthy people (Reihelt, Barz, and Thude,
2009) are remarkable in a sense of possible influence of such variability on individual resistance to diseases and malignancy. Taking into account recent “scandal” in the field of oncology after information about lowest benefit of cytotoxic chemotherapy (Morgan, Ward, and Barton, 2004; Begley and Ellis, 2012) the additional purposeful investigations are necessary to understand partially how exhaustion of haematopoiesis relates to death event of cancer patients.

From this point of view, very attractive puzzle is an essential variety, disorder of results of treatment by the criterion of life span even among patients with identical diagnostic characteristics of malignant process, and, hence, receiving identical treatment. This diversity manifests itself in the sloping form of survival curves and in existence of widely varying individual life spans. Earlier we showed a big latent heterogeneity of prognosis among cancer patients with uniform diagnosis approximating their survival curves by two exponents with very different values of exponential rate (Bochkareva, Ekimova, Nemkova, Sokurenko, and Shutko, 2008). Then we investigated the data, extracted from premier source for cancer statistics “Surveillance, Epidemiology and End Results” Program (USA), and found that monotonous long-term declining of survival according exponential principle is accompanied by regular short-term fluctuations with different frequency, depending on the level of survival on 3rd-4th years since the therapy start (Shutko, Akushevich, Ekimova, Karamullin, and Yashin, 2008).

The purpose of the present work was the comparative study of dynamic relations between the fluctuation of survival rate and presence of hematopoietic progenitor cells in circulating blood of oncologic patients in the final months of their life.

**Method**

The objects of research: 37 patients with late stages of cancer of oral-pharyngeal area (OPhC) and 33 with non-small cells lung carcinoma (NSCLC) received conventional chemo-radiotherapy in Russian Research Center of Radiology and Surgical Technologies, in Saint-Petersburg. Research was made with the informed consent obtained from participants, within 1.1 year after the beginning of therapy and included:

a) monitoring of patients survival with an average frequency of 1 time each 0.5 months (NSCLC) or each 2 months (OPhC) until 0% survival of NSCLC patients at 10th month and 43% survival of OPhC patients at 13th month

b) parallel monitoring of stem/precursors CD34+cells in circulating blood of patients with frequency of inspection each 0.5 months (NSCLC) or each 1 month (OPhC). Flow cytometric measurements were performed on a FACScan flow cytometer (Becton Dickinson), using a two-color analysis with anti-human CD34-RPE antigen (clone: Birma-K3; this antibody reacts with the protein expressed on immature haematopoietic stem/progenitor cells and capillary endothelial cells, Dako Cytomation, Denmark A/S) and with auxiliary anti-human CD45-FITC (clone: T29/33 to common leukocyte antigen, Dako Cytomation). In detail, 5 ml of peripheral venous blood anticoagulated with heparin were taken to identify circulating CD34+ cells. Mononuclear cells were isolated by centrifugation on separation medium (1.077 g/ml ficoll-verografinum. Then the cells were washed twice with PBS, pH 7.3 (Sigma), cooled at 6 °C and labeled with 10 µl anti human CD34-RPE antibody and 10 µl anti-human CD45-FITC antibody (in proportion1/10) for 30 minutes in the dark. Afterwards, the cells were washed again twice with PBS and in volume 0.5ml of 0.01mol/L. PBS were analyzed on cytometer using for each sample the lateral and forward scattering limits, restricting zones of lymphocytes and monocytes. About 400,000-300,000 events were registered for each sample, and the calculated result was measured as percent of CD34+ cells in total mononuclear cells subset.

All data were analyzed retrospectively next way:

**Stage 1.** Two curves of survival were approximated by exponents in the “Microsoft Excel”
Medical and Health Science Journal / MHSJ / ISSN: 1804-1884 (Print) 1805-5014 (Online)

program and were characterized in terms of exponential death rate \( k(\text{month}^{-1}) \) in equation (Bochkareva et al., 2008):

\[
S_t = Ae^{-kt} + Be^{-kt'}
\]  

(1)

where \( t \) - time elapsed since therapy’s start, months, \( S_t \) - current survival at elapsed time \( t \), relative units, \( A \) and \( B \) - parts of patients dying with corresponding exponential rate of death \( k \) or \( k' \), relative units. \( S_t = 1.0 \) at \( t = 0 \).

According parameter of monotonous rate of death all patients were divided retrospectively into three categories: dying with a slow rate \( k = 0.0633 \) month\(^{-1}\) (OPhC patients), with intermediate rate \( k = 0.125 \) of month\(^{-1}\) (NSCLC patients), and with speedy rate \( k = 0.31 \) month\(^{-1}\) (NSCLC patients). The coefficients of correlation for corresponding tree exponents are \( R = 0.996 \pm 0.0806 \) (p<0.001), \( R = 0.957 \pm 0.054 \) (p<0.001) and \( R = 0.796 \pm 0.214 \) (p=0.006). The dynamic parameters “\( k' \)” in a given investigation phase permitted us to evaluate preliminarily average rate of monotonous declining of survival in each of two groups. For evaluation of fast fluctuations of death rate, the proportions \( \{P\} \) of the dead for short identical consecutive periods in each one of two groups (OPhC and NSCLC) were calculated as ratio: number of dead in period/number of patients in the whole group. The proportions \( P \) were presented depending on average life span for those who was gone during corresponding consecutive period. Zero time since the therapy starting designates zero point on the scale of life span.

**Stage 2.** In parallel, the average percent of CD34+ cells in total mononuclear cells' subset was calculated for those who stay yet alive during corresponding consecutive period since the therapy start and will die later, at his own zero time.

**Stage 3.** The comparison of average percent of CD34+ cells in the blood of the patients, who are still living, with the proportion of dead patients at different distance named life span between the current period and the case of death named zero time point.

The comparison was performed by formal description and statistical estimations of kinetic dependences for percent of CD34+ cells and proportion of dead patients using approximations by various periodic functions in programs “Microsoft Excel” and Vernier Graphical Analysis (2002) with calculation of correlation coefficients \( R \), their errors \( m_R \) and the probabilities of differences \( p \). The results obtained with the program “Vernier Graphical Analysis” are described in the text, though not shown in the form of graphs because of their similarity to the results of the program “Microsoft Excel”

**Results**

Figure 1 presents the results of approximation of the data received for OPhC.

The existence of percentage fluctuations of CD34+ cells is confirmed by significant \( R \) in approximated line formula:

\[
\%CD34 = 6E-05x^5 - 0.0023x^4 + 0.0286x^3 - 0.141x^2 + 0.2502x + 0.0005, \quad (2)
\]

where \( x \) - life span (months); \( R = 0.81 \pm 0.14; \ p < 0.001 \).

The fluctuation of proportion of the dead (\( P \), relative units) were found also not monotonous:

\[
P = 0.0002x^4 - 0.0049x^3 + 0.0353x^2 - 0.0598x + 0.1243, \quad (3)
\]
where \( R = 0.94 \pm 0.16; p=0.02 \)

**Figure 1. The comparison of dynamics of %CD34+ cells in the blood of living patients and proportion of dead ones with OPhC**

The approximation of points on Figure 1 was made in the Vernier Graphical Analysis (2002) by harmonious function (a sinusoid):

\[
y = A \times \sin(B \times x + C) + D
\]

(4)

where \( y \) is the % of CD34+ cells or proportion of dead P, the \( x \) is the life span (months), and \( B \) is a parameter of frequency \( =2\pi/T \), were \( T \) is the period of oscillation (months). Here, \( B=0.548 \pm 0.0411 \) for %CD34+ cells and \( 0.531 \pm 0.0823 \) for proportions \( P \), what corresponds to length of periods 11.5 and 11.8 months.

The maximums on Figure 1 are shifted each relatively other, and maximum of death seemed to follow the maximum CD34+ on the scale of shortening life span \( x \) toward time zero that is death.

Data for patients with NSCLC lead to the similar conclusion (Figure 2).

The fluctuation of % CD34+ cells described by formulas:

\[
\%CD34=0.0102x^4 - 0.2966x^3 + 3.1381x^2 - 14.307x + 23.984
\]

\[
R = 0.94 \pm 0.12; p<0.001
\]

(5)

with frequency \( B=-1.29 \pm 0.15 \) for Figure 2B or

\[
\%_{CD34} = -0.0098x^6 + 0.1875x^5 - 1.3995x^4 + 5.1434x^3 - 9.5816x^2 + 8.2659x - 2.195
\]

\[
R = 0.85 \pm 0.18; p=0.002
\]

(6)

with frequency \( B=-2.96 \pm 0.15 \) for Figure 2A.
The proportion of death P described by formulas:

\[ P = -0.0001x^6 + 0.008x^5 - 0.1833x^4 + 2.174x^3 - 14.112x^2 + 47.539x - 64.921 \]

\[ R = 0.79 \pm 0.19; \ p=0.002 \]

(7)

with frequency B=1.88±0.221 for Figure 2B or

\[ P=-0.012x^6 + 0.1855x^5 - 1.1016x^4 + 3.1498x^3 - 4.442x^2 + 2.8224x - 0.5707 \]

\[ R=0.95 \pm 0.11; \ p<0.001 \]

(8)

with frequency B=3.23±0.25 for Figure2A.

According to parameters B the periods of fluctuations of %CD34 and proportion of death P on Figure 2B make correspondingly 4.7 and 3.3 months, on Figure.2A they are shorter - 2.1 and 2 months.

**Discussion**

Relatively small amount of the investigated patients reflects technical and ethic difficulties in the organization of timely getting of the blood's samples from those who still alive. Nevertheless, the three ranges of data were received for: the average exponential rate of death (0.0633, 0.125 and 0.31 months⁻¹), the period of fluctuations of %CD34+ cells (11.45, 4.7 and 2.12 months), and for the period of fluctuations of death proportion P (11.83, 3.3 and 1.96 months). Thus, the faster average rate of death (k), the longer common period of fluctuations for both parameters (T).This regularity can be described using all mentioned points as

\[ T = 0.5899 \times k - 1.0516; \ R = 0.97 \pm 0.13; \ p=0.002 \]

(9)

This equation, on the one hand, confirms our former conclusion relative to the fluctuations of monthly mortality rate (Shutko et al., 2008). On the other hand, it
discovers dependence of these fluctuations from fluctuations of CD34+ cells’ pool in circulation. The increase of signs of instability of functioning of the haematopoietic system in the form of augmentation of frequency and amplitude of fluctuations of cellular structure of a blood (so-called “turbulent” haematopoiesis) occurs mainly during the premortortal periods of time in the conditions of long (from the moment of a birth up to death) irradiation of the total organism of mammals in small daily doses of ionizing radiation (Fliedner and Graessle, 2008). Taking into account the opposite phases of two parameters on Figures 1 and 2 we believe that the increased risks of death arise periodically among patients with the maximum concentration CD34+ cells in a blood. It doesn’t contradict data on bad prognosis at randomized registration of the increased quantities of stem cells in a blood of patients with malignancy (Itinuma, Watanabe, Mimori, Adachi, Hayashi et al., 2011; Yu, Lin, Yan, Tian, Li, and Lin 2011; Armstrong, Marengo, Oltean, Kemeny, Bitting, Turnbull et al., 2011; Goon, Lip, Stonelake, and Blann 2009). The data opposite than cited ones occur less frequently (Fan, He, Liu, Zhu, Liu, et al., 2011). As the about 60% of the CD34+ cells carry marker CD133, being primitive progenitor cells with angiogenic properties (Reihelt et al., 2009), negative influence of ascending of the CD34+ cells in the form of the delayed rising of the current proportion of dead patients could be explained in our case by a periodic activation of neoangiogenesis inside of the tumor tissues. The tumor angiogenesis many times surpasses the one in histologically identical normal tissue (Yamaura and Matsuzawa, 1979). The fact is considered as the principal cause of the malignancy progression, as well as recognized as a main target for the new type of tumors treatment, so-called antiangiogenic therapy (Heymach, Folkman, Kalluri, Deker, 2010; Folkman, 2004).

The other or concomitant explanation version for the received data refers to the periodic weakening of the ability of circulating haematopoietic stem cells to support cell’s renewing in normal tissues and organs, which are critical for life support (Shoutko and Shatinina, 1998; Kucia et al., 2005; Drapeau, 2010). In any case, a clear time correlation of two parameters (rate of death and CD34+ stem cells concentration in the blood) proves the existence of variable conditions for successful realization of the chosen therapy.

**Conclusion**

The registered associativity turbulence in the content of CD34+ cells in a blood of the advanced oncologic patients and turbulence of probability of their death leads to the assumption that the reason for life’s cessation may be the abnormal morphogenic activity of young cells produced in the bone marrow toward the tumor tissues of the host, as well as a lack of such activity toward the critical normal tissues. Thus, the cytotoxic, especially long-term, therapy of patients with advanced cancer may be timely even harmful for them and compromise the ultimate result of treatment. The regimes of cytotoxic therapy of patients in cases of premature exhaustion of haematopoietic resource should be optimized based on individual periodicity of blood saturation with progenitor cells. The development of reliable and simple methods for clinical monitoring of quickly changing prognosis of patients status seems to become important aim of coming investigation.

**References**


Drapeau, Ch., 2010. Cracking the Stem Cell Code, US: Sutton Hart Press


Shoutko, A., Shatinina, N., “Chronic cancer - could it's be?,” 6 Annual Conference of MSAIMA, November 25, 1998, Tel-Aviv


Vernier’Graphical Analisis, 3.1 DemoVersion, Texas Instruments. 2002


