

longitudinal sections were made. The stain used for histology was Alcian&Alizarin.

Results: The development of long bones in vitamin C deficient guinea pigs are considerably stagnant. Hyaline cartilage models are significantly shortened. Ossification in the diaphyses of carpal and metacarpal bones are absent, and the organization of the epiphyseal plates is very irregular with the reduction of number of chondrocytes. Moreover, there are numerous haemorrhagic regions and subperichondrial bleeding with separation of perichondrium.

Conclusion: Deprivation of vitamin C during inrauterine period disables normal development of long bones. Disorder of hyaline cartilage models was seen, as well as the disorder of ossification.

<http://dx.doi.org/10.1016/j.pbj.2017.07.085>

PS060

Peculiarities of expression of apoptosis markers in the tissues of primary fallopian tubes carcinoma



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Aim: immunohistochemical analysis of apoptosis markers in the tissue of PFTC.

Introduction: Primary fallopian tubes carcinoma is a rare case among oncological diseases of female genital organs, but the mortality rate is rather high. Nowadays, the prognostic factors of this neoplasia are not fully determined. The data on the p53 and bcl2 proteins expression and their use as prognostic factors in patients with malignant tumors of many locations are contradictory.

Methods: the study was conducted on 66 samples of fallopian tubes tumor tissue. To study the apoptosis peculiarities of tumor cells the mouse monoclonal antibodies for bcl-2 (clone 100/D5) and p53 (clone SP5) were used. Mathematic calculations were done using Microsoft Excel 2010 with AtteStat 12.0.5.

Results: The high expression of p53 was found in patients of all clinical stages. Mutations of p53 increased with spreading of the neoplastic process. Strong correlation of p53 presence in tumor samples and clinical stage of the disease was determined ($r=0.77$). In contrast to the abovementioned protein the study of bcl-2 showed the moderate negative correlation between this protein and the stage of the disease ($r=-0.54$). Analysis of the dependence of p53 expression with the presence or absence of lymph nodes metastasis showed a direct correlation between the indicators ($r=0.25$). Thus the level of p53 expression in patients with N1 was $80.6 \pm 2.7\%$ compared with the N0 group ($29.7 \pm 3.6\%$). The stage of neoplasia differentiation is in moderate direct correlation with p53 expression ($r=0.58$) and in inverse with – bcl-2 ($r=-0.64$).

Conclusion: Expression of p53 depends on neoplasia spreading and stage of tumor differentiation. The expression of p53 is an independent prognostic marker for N-status and helps to classify the patients into “risk” groups.

Acknowledgements: Supervisor: A. M. Romaniuk, prof., doctor of medical sciences, Department of Pathological Anatomy, Medical Institute, Sumy State University.

<http://dx.doi.org/10.1016/j.pbj.2017.07.086>

PS064

Analysis of combined impact of doxorubicin and menadione on human leukaemia Jurkat T cells



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Aim: The anti-proliferative effect and the mechanism of action of doxorubicin(DOX) in combination with menadione(MD) were studied in Jurkat T cells, a model for acute lymphoblastic leukaemia (ALL).

Introduction: Doxorubicin is a well-characterized and successful antineoplastic drug commonly used in various cancer treatments, including ALL. Menadione has proven a strong proapoptotic effect in Jurkat cells.¹⁻³

Methods: Cell cycle, apoptosis/necrosis and the oxidative status were assessed by flow cytometry on propidium iodide, Annexin V-FITC/PI and CM-H2DCFDA/7-AAD labelled cells, respectively.

Results: Oxidative stress induced within 4h by MD ($IC_{50}=11.5 \mu M$) was reduced in the presence of 500 nM DOX ($IC_{50}=22.0 \mu M$). After treatments of 18 h, DOX induced cell cycle arrest displaying a trimodal distribution; successive G2/M, S and G0/G1 blockage was produced with an IC_{50} of 49 nM, 464 nM and 1866 nM, respectively, whereas in the presence of 7.5 μM MD, increasing levels of DOX mainly induced S-phase arrest. Within 18 hours of exposure, DOX induced apoptosis in a biphasic dose-dependent manner ($K_d=335$ nM and 3.29 μM , respectively). Addition of 7.5 μM MD enhanced apoptosis at <300 nM DOX, but reduced cell death at higher levels of DOX. However, 48 h after drug removal the apoptotic rate was considerably higher in cells exposed to DOX:MD, which also showed consistent fractions of early apoptosis (up to 44%). The efficacy of DOX was doubled by MD($K_d=46.5$ nM in the presence, and $K_d=99$ nM and 143 nM in the absence of MD).

Conclusion: Data indicate that clinically relevant levels of MD and DOX in combined treatments can exert considerable cytotoxic impact on Jurkat cells, via cell cycle arrest and apoptosis induction. These findings could encourage new therapeutic strategies to improve the therapeutic index of doxorubicin in ALL treatments.

Acknowledgements: This work was supported by a fellowship of the Romanian Ministry of Education, UEFISCDI, for Young Researchers, project number 8/2016.

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<http://dx.doi.org/10.1016/j.pbj.2017.07.087>

PS068

Effect of symptom interval and demographic characteristics on initial stage of malignant tumors in children



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Aim: The aim of our retrospective study was to determine the influence of demographic and clinical characteristics of patients, initial stage of disease and tumor size on symptom period in children with malignant tumors.

Introduction: One of the main goals in pediatric oncology is timely diagnosis, cause it allows prompt and more effective treatment and significantly decreases the number of complications. The majority of children with malignant tumors have specific or non-specific symptoms certain time period before the diagnosis which can point towards malignant disease.

Methods: Our study included 296 children with malignant tumors, diagnosed and

treated between 2005 and 2016 in University Children's Hospital in Belgrade. Collected data included sociodemographic parameters, variety of symptoms and its duration, initial stage of disease and size of the tumor.

Results: The most frequent tumors were as follows: neuroblastoma, Hodgkin and non-Hodgkin lymphoma and kidney tumors. Non-Hodgkin lymphoma was diagnosed more frequently in boys, while Ewing sarcoma and primitive neuroectodermal tumors were seen mostly in girls. The majority was admitted at IV stage (30.1%) in opposite to 13.5% of patients in I stage. The average symptom interval was 87.7 days (median 46; SD= 164), from 5 to 2190 days. We have proven that following factors have significant effect on the extent of symptom interval: age ($p < 0.001$), type of tumor ($p < 0.05$), its localization ($p < 0.001$), specific symptoms ($p < 0.05$), and referral from primary health care unit in comparison to secondary one ($p < 0.05$).

Conclusion: The results of our study give a new insight in symptom interval of children with malignant tumors in our country. More detailed comprehension of patients' characteristics, their diseases, healthcare system and their effect on symptom interval could significantly contribute to early diagnosis, as well as decreased number of complications at admission and during treatment.^{1–6}

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<http://dx.doi.org/10.1016/j.pbj.2017.07.088>

PS069

Impact of prior malignancies on the outcome of colorectal cancer: Revisiting clinical trial eligibility criteria



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Aim: To study the impact of prior malignancies on the survival of subsequent CRC.

Introduction: Colorectal cancer (CRC) is the third most common cancer in the US.^{1–3} Some studies have correlated a prior history of malignancy with an increased incidence of CRC. Patients with history of cancer are generally excluded in clinical trials. This practice, not only affects clinical trials accrual, but also limits the potential therapeutic options for this population. The rationale behind this exclusion is that a history of malignancy could potentially interfere with the study outcomes.⁴ However, little is known about its real impact on survival of subsequent CRC.

Methods: We identified patients with CRC diagnosed between 1973 and 2008 using the National Cancer Institute's SEER database.^{5,6} Outcomes of interest were overall survival and cause-specific survival of subsequent CRC in general, and specifically stage IV disease. Unadjusted Kaplan-Meier test and multivariable covariate-adjusted Cox models were used to assess the eligibility of enrollment of stage IV CRC patients in clinical trials.

Results: Overall, 550,325 patients with CRC were identified, of whom 31,663 patients had a prior malignancy. Both, history of prior non-leukemic malignancy and prior leukemia were associated with a worse overall survival (HR = 1.165 95% CI = 1.148–1.183, $P < 0.001$) and (HR = 1.825 95% CI = 1.691–1.970, $P < 0.001$), respectively. However, a history of any prior non-leukemic malignancy showed a favorable colorectal-specific survival (HR = .930 95% CI = .909–.952, $P < 0.001$). Analysis of stage IV CRC showed that a history of any prior non-leukemic malignancy was not associated with a significant difference in overall survival but having a history of leukemia showed a worse overall survival (HR = 1.535, 95% CI = 1.303–1.809, $P < 0.001$).

Conclusion: Clinical trials should take these results into consideration when including/excluding stage IV CRC patients with prior malignancies.

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<http://dx.doi.org/10.1016/j.pbj.2017.07.089>

PS071

Intervention of diabetes mellitus and metabolic risk factors in AMPK-PGC1 α -SIRT3 pathway in the human corpus cavernosum



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