Alpha-fetoprotein, carcinoembryonic antigen and beta2-microglobulin in adult population highly exposed to organochlorinated pollutants (PCB, DDE and HCB)

[1]Langer P, [2]Kocan A, [2]Drobna B, [1]Huckova M, [1]Radikova Z, [3]Susienkova K, [1]Imrich R, [1]Ukropec J

[1]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; [2]Dept. of Toxic Organic Pollutants, Slovak Medical University, Bratislava, Slovakia; [3]Dept. of Statistics, Faculty of Economic Informatics, Economics University, Bratislava, Slovakia
e-mail: pavel.langer@savba.sk

Objective. It is aimed to obtain some general information about the prevalence of certain biomarkers in highly exposed population and on the interrelations between their serum level as related to that of some major organochlorines (OCs).

Methods. The level of alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA) and beta2-microglobulin (beta2-MG) as well as that of polychlorinated biphenyls (Σ15PCBs), dichlorodiphenyl-dichloroethylene (DDE) and hexachlorobenzene (HCB) was estimated in 2046 adults (834 males and 1212 females) from highly polluted Eastern Slovakia.

Results. Great majority of blood levels was lower than two specific units used for individual markers, while the prevalence of values higher than two specific units of appropriate markers. At the same time, the prevalence of all markers level higher than 2 specific units was highly significantly increasing with of stratified PCBs level quintiles which were also positively related to these of DDE and HCB. Some significant correlations between biomarkers level and age were also observed.

Conclusions. Although from the data obtained within this multipurpose field survey any notable interrelations between AFP, CEA and beta2-MG and some specific diseases and/or malignant processes could not be retrospectively specified, from the data obtained it appears that some of such interrelations cannot be definitely excluded.

During previous twenty years great attention has been paid to the population living in Eastern Slovakia which was exposed to high environmental pollution by several organochlorines (e.g. PCBs, DDE and HCB) resulting from environmental negligence of former totalitarian regime (Kocan et al. 1994, 2001; Chovancova et al. 2005; Petrik et al. 2006; Jursa et al. 2006; Hovander et al. 2007; Linderholm et al. 2007; Park et al. 2007, 2008). In addition to several previous findings on increased thyroid volume, thyroid hormone and thyroid antibodies level with increasing level of organochlorines (Langer et al. 2007a, 2007b, 2007c, 2010) also increased prevalence of prediabetes and diabetes in polluted areas has been recently reported (Ukropec et al. 2010).

The aim of this report is to summarize the observations on the interrelations between the level of those organochlorines mentioned above and some biomarkers such as alpha-fetoprotein, carcinoembryonic antigen and beta2-microglobulin which were selected because of their possible interrelations with some malignant processes.

Subjects and Methods

Subjects. A total of 2046 adults from Eastern Slovakia was examined consisting of 834 males and 1212 females (578 aged 19-40 years and 1468 aged 41-78 years), a part of them (432 males and 576 females) being recruited from the district of Michalovce which has been previously found heavily polluted by polychlorinated biphenyls (PCBs) and other organochlorines, namely by dichlorodiphenyl-dichloroethylene (DDE) as well as hexachlorobenzene (HCB) for several decades. Remaining subjects (402 males and 636 females) were

Table 1

Distribution of organochlorine and AFP levels in females and males as sorted in terms of Σ15PCB level quintiles

Gender	Quintile of Σ15PCB level	N	Means ng/g serum lipid			Number AFP (ng/ml)		
			PCB	DDE	HCB	>2.0	>5.0	>10.0***
Females*	1	243	618	2250	618	85	0	0
	2	242	985	1723	985	128	24	1(2)
	3	242	1021	2224	1021	141	34	3(0)
	4	243	1215	3130	1215	135	16	2(1)
	5	243	1607	3774	1607	165	47	7(0)
TOTAL		1213			TOTAL	654	121	13 (3)
Males**	1	166	621	1094	378	69	2	0
	2	167	976	1657	561	79	8	1
	3	167	1349	2271	605	100	17	1
	4	167	3042	3297	773	103	13	0
	5	167	5424	3978	1001	151	20	1
TOTAL		834			TOTAL	502	60	3

Chi-square for quintiles of PCB and AFP >2.0 ng/ml: * = in females, chi-square = 94.98, p<0.0000 ;** = in males, chi square = 106.64, p<0.0000; *** = explanation: among a total of 16 females with AFP level >10.0 IU/ml three were pregnant (with AFP level of 26.7, 74.7 and 103.7 IU/ml and average age of 22 years), their number being shown in brackets, while the average age of additional 13 females with that level range was 53 years (range of 48-71)

from the districts of Svidnik and Stropkov located about 60 kilometers upwind and upstream and considered as exposed to background pollution appropriate to east Slovakian area.

The subjects were recruited by 28 a priori selected primary care local physicians to which all residents were assigned as based on their residence. They have been instructed to divide the alphabetical list of subjects under their care into centiles and then to call randomly one subject per each centile to fulfil the final aim to recruit about 30 percent of subjects <40 years and 70 percent between 41 and 75 years, while the ratio of males to females should be kept about 40 to 60 percent. By such a way about 60 to 100 subjects were finally recruited by each physician.

Examinations. The examination consisted of questionnaire data, physical examination and informed consent document. The procedure was approved by Institutional Review Board and by anonymous reviewers of European Commission.

Blood was withdrawn by the vacutainer and centrifuged in a refrigerated centrifuge. Serum aliquots were then transported in portable freezer to the laboratory and kept frozen at -20 °C until assayed.

Estimation of organochlorines. From each subject 20 ml of blood was withdrawn and centrifuged

in a refrigerated centrifuge. The aliquots of serum and urine were transported in portable freezer to the laboratory and kept frozen at -20 °C until assayed. In serum of all subjects fifteen PCB congeners (IUPAC numbers 28, 52, 101, 105, 114, 118, 123, 138[+163], 153, 156[+171], 157, 167, 170, 180 and 189) and also p,p'-DDE (2,2'-bis(4-chlorophenyl)-1,1-dichloroethylene), p,p'-DDT (2,2'-bis(4-chlorophenyl)-1,1,1-trichloroethane), hexachlorobenzene (HCB) as well as α-, β- and γ-hexachlorocyclohexane (HCH) were determined by high resolution gas chromatography and Ni-63 microelectron capture detector using a 60 m DB-5 capillary column as repeatedly described elsewhere (Kocan et al. 2001; Pavuk et al. 2004; Petrik et al. 2006).

Estimation of biomarkers. Serum level of alphafetoprotein (AFP), carcinoembryonic antigen (CEA) and beta2-microglobulin (beta2-MG) was estimated by highly sensitive electrochemiluminiscent immunoassay using the automatic system Elecsys (Roche, Germany).

Treatment of data end statistical evaluation. For each biomarker the data obtained for males and females were treated separately. After being divided in terms of stratified PBC level quintiles, the prevalence of data for each biomarker belonging to individual PCB quintiles were further sorted in terms of three cut-off levels (e.g.

Table 2

Distribution of organochlorine and CEA levels in females and males as sorted in terms of Σ15PCB level quintiles

Gender	Quintile of Σ15PCB level	N	Means			Number		
			ng/g serum lipid			CEA (ng/ml)		
			PCB	DDE	HCB	>2.0	>5.0	>10.0
Females*	1	243	618	2250	618	39	4	0
	2	242	985	1723	985	50	1	0
	3	242	1021	2224	1021	65	5	0
	4	243	1215	3130	1215	63	10	0
	5	243	1607	3774	1607	80	10	2
TOTAL		1213			TOTAL	297	30	2
Males**	1	166	621	1094	378	65	7	0
	2	167	976	1657	561	36	8	0
	3	167	1349	2271	605	74	11	0
	4	167	3042	3297	773	75	15	2
	5	167	5424	3978	1001	122	23	0
TOTAL		843			TOTAL	372	64	2

Chi square for quintiles of PCB and CEA >2.0 ng/ml: * in females, chi-square = 29.52, p<0.0003; ** in males, chi square = 96.59, p<0.0000

>2, >5 and >10 specific units used for each biomarker) and presented in separated tables for each biomarker. Statistical significance showing the interrelation between the level of PCB and prevalence of all levels >2 units was evaluated by chi-square test.

Results

In both males and females, with the aid of chi-square analysis highly significant increase in the prevalence of values >2.0 ng/ml for AFP (Table 1) as well as these >2.0 ng/ml for CEA (Table 2) and these >2.0 mg/l for beta2-MG (Table 3) with increasing level of Σ15PCBs as stratified in quintiles has been found. Moreover, each of these Tables shows a considerable increase of DDE and HCB level which was parallel to that of Σ15PCBs. From this follows that all major organochlorines analyzed could participate in the mechanisms and/or pathological processes which are hidden behind such increased level of those markers.

Although the presented chi-square analysis for all biomarkers was related only to the sum of values >2.0 appropriate units used for each of them, all Tables also show considerably increasing and parallel prevalence of the values >5.0 appropriate units, namely for AFP and CEA.

However, some data showing biomarker levels in general population without some "marker producing disorders" still seem lacking. Nevertheless, there still was a considerable number of values <2.0 appropriate units which could certainly belong to such unaffected population. Thus, in our cohort such prevalence of "normal" AFP level was 559/1213 (46.1 %) in females and 332/834 (39.8 %) in males (Table 1), while much higher prevalence of such "normal" level was found for CEA, e.g. 916/1213 (75.5 %) in females and 584/834 (70.0 %) in males (Table 2) as well as for β2-MG, e.g. 924/1213 (76.3 %) in females and 584/834 (70.0 %) in males (Table 3).

Discussion

Alpha-fetoprotein (AFP) is a glycoprotein, the highest amount of which occurs in the human fetus. It does not have any known function in adult humans, but increased AFP level appears in pregnant women due to its production by fetus. However, increased level occurs in patients with AFP secreting tumors such as endodermal sinus tumor (yolk sac carcinoma), neuroblastoma, hepatoblastoma, hepatocellular carcinoma and nonseminomatous germ cell tumors. It may be also increased in patients with chronic benign liver disease (cirrhosis, hepatitis).

Shin et al. (2010) observed 66 patients with hepatocellular carcinoma (HCC) who had preoperative AFP level >400 ng/ml and found postoperative recurrence

Table 3

Distribution of organochlorine and beta2-MG levels in females and males as sorted in terms of Σ15PCB level quintiles

Gender	Quintile of Σ15PCB level	N	Means			Number		
			ng/g serum lipid			beta2-MG (µg/ml)		
			PCB	DDE	HCB	>2.0	>3.0	>5.0
Females	1	243	618	2250	618	37	2	–
	2	242	985	1723	985	50	1	--
	3	242	1021	2224	1021	54	1	–
	4	243	1215	3130	1215	72	3	–
	5	243	1607	3774	1607	76	8	1
TOTAL		1213		TOTAL		289	15	1
Males	1	166	621	1094	378	30	1	–
	2	167	976	1657	561	45	3	–
	3	167	1349	2271	605	48	3	1
	4	167	3042	3297	773	63	1	–
	5	167	5424	3978	1001	64	4	2
TOTAL		834		TOTAL		250	12	3

Chi square for quintiles of PCB and beta2-MG >2.0 µg/ml: * in females, chi-square = 33.74, p<0.0000;
** in males, chi square = 27.47, p<0.0006

only in those with AFP increase above 20 ng/ml mostly within one year. In contrast, Jiang et al. (2011) recommended 10 ng/ml as the best cut-off level for diagnosis of primary HCC. Mailey et al. (2011) stratified a total of 2253 patients with elevated AFP level before orthotopic liver transplant for hepatocellular carcinoma into low AFP (1210 patients with AFP <20 ng/ml), medium AFP (855 patients with AFP 20-399 ng/ml) and high AFP (238 patients with AFP >400 ng/ml) group, the medium and high AFP groups being associated with higher mortality.

However, Chen et al. (2009) reported 102 consecutive subjects with a histological diagnosis of chronic hepatitis C without any presence of hepatocellular carcinoma for at least 6 months' follow-up, but with hepatic steatosis (> or = 5 % hepatocytes), hepatic fibrosis (> or = stage II), uric acid (> or = 6.3 mg/dl), asparate aminotransferase (> or = 40 IU/l), albumin (< 3.5 g/dl), and fasting plasma glucose (<126 mg/dl), all these findings being significantly associated with elevated AFP (> or = 13.6 ng/ml) in 29 among those patient. Similarly, increased AFP level (>15.0 ng/ml) was found in 23.9 % among 654 similar patients (Tai et al. 2009) and thrombocytopenia, aspartate aminotransferase and alanine transaminase elevation and AFP level >6 ng/ml were associated with advanced fibrosis. Among 357 US patients with chronic hepatitis C 23.0 % had elevated serum AFP (> or = 10 ng/ml) that was independently associated with

stage III/IV hepatic fibrosis, elevated level of aspartate aminotransferase and prolonged prothrombin time (Hue et al. 2004).

From the above findings it appears that the increase of AFP occurs not only in cases with hepatocellular carcinoma, but also in cases of chronic hepatitis C without any presence of such carcinoma. Considerable number of such patients had AFP level >15.0 ng/ml, though the level of 10 ng/ml has been also suggested as cut-off level for diagnosis of HCC (Jiang et al. 2011). In addition, Tai et al. (2009) showed that the elevation of AFP >6 ng/ml has been associated with advanced fibrosis.

However, some data showing AFP level in general population without AFP producing disorders still seem lacking. The data found in our cohort (Table 1) shows 559/1213 (46.1 %) AFP levels <2.0 ng/ml in females and 332/834 (39.8 %) in males, while the prevalence of AFP levels <5.0 ng/ml in females was 1092/1213 (90.5 %) and that in males was 774/834 (92.3 %). In contrast, the same Table 1 shows 12/1213 (0.98 %) cases with AFP >10 ng/ml in females and 3/834 (0.35 %) of such cases in males and highly significant increase of AFP values >10 mg/l with AFP level >2 mg/l with PCBs.

Carcinoembryonic antigen (CEA) is a type of protein molecule that is associated with some cancers of gastrointestinal tract, but also with those of breast, lung, and certain types of thyroid and ovarian cancer. Benign conditions which can elevate CEA include smoking, in-

fections, inflammatory bowel disease, pancreatitis, liver cirrhosis and some benign tumors in the same organs in which an elevated CEA indicates cancer.

The normal range for CEA in adult non-smokers is <2.5 ng/ml, but it may be higher in smokers (<5.0 ng/ml). The discriminative value of 5.0 ng/ml has been recently supported by the observation of 4509 subjects referred to large bowel endoscopy due to the symptoms of colorectal cancer, the odds ratio for cancer being found highly significant in those with CEA levels >5 ng/ml, while in those with CEA level <5 ng/ml it was not (Nielsen et al. 2010). However, the levels >20 ng/ml as found before therapy are associated with the cancer which has already spread.

In the recent review (Tan et al. 2009) also the question of diagnostic precision of serum CEA level in the detection of local or distant recurrence following resectional surgery for colon and rectal cancer has been thoroughly studied by quantitative meta-analysis in 20 studies in a total of 4288 patients. The cut-off for a "positive" CEA ranged from 3 to 15 ng/ml between various studies, but the optimum cut-off level for the best combination of sensitivity and specificity was 2.2 ng/ml which has been considered an ideal balance of sensitivity and specificity and also which may be useful as a first-line surveillance investigation in patients during surgical follow-up based on serial CEA measurements using temporal trends in conjunction with clinical, radiological and/or histological confirmation.

From these findings it appears that the cut-off level of 2.0 ng/ml used in our survey is close to that recommended (Tan et al. 2009) and that highly significant increase in the prevalence of CEA levels >2.0 ng/ml with the level of PCB and other organochlorines (Table 2) could support the view on their participation in the development of CEA related disorders.

In our cohort (Table 2) we found 916/1213 (75.5 %) CEA levels <2.0 ng/ml in females and 584/834 (70.0 %) in males, while only few values in females (30/1213 = 2.5 %) and in males (64/834 = 7.6 %) were >5.0 ng/ml.

Serum level of β2-microglobulin (β2-MG) belongs to the markers of survival in patients with multiple myeloma and lymphoma (Xu et al. 2010) and chronic lymphocytic leukaemia (Zenz et al. 2010). Among 159 patients with acute lymphocytic leukemia the β2-MG level >4.0 mg/l has been found in patients with significantly worse survival (Kantarjian et al. 1992). Nevertheless, also somewhat lower β2-MG level such as of 3.0 mg/l appeared predictive for the transformation of lymphoma to follicular lymphoma in 34 patients among a total of 220 patients (Bastion et al. 1997).
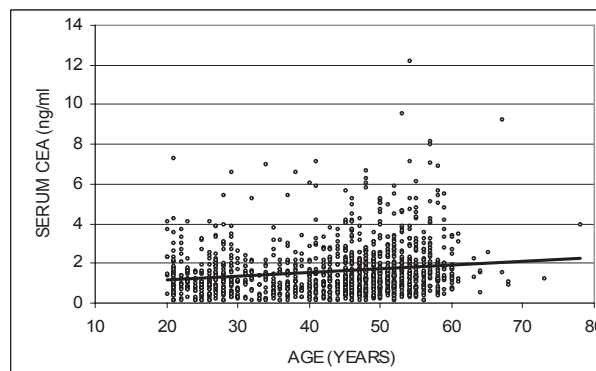

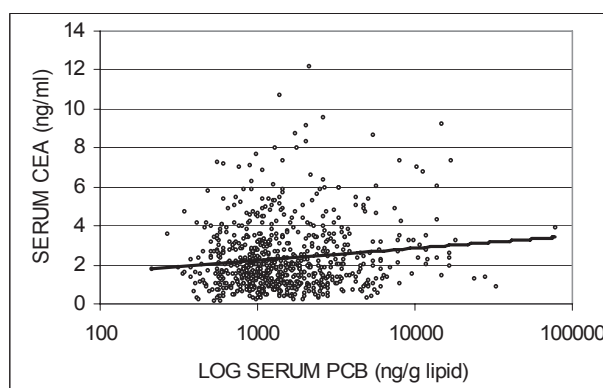Fig. 1 Significant correlation between the age and CEA level in all subjects (r = 0.034; p<0.05|

Fig. 2 Significant correlation between the level of PCBs and CEA in all subjects (r = 0.110; p<0.001|

Greipp et al. (1993) reported the survival of 28 months for multiple myeloma patients with both C reactive protein and β-MG >6 mg/l, while the survival for those with both levels <6 mg/l was 47 months.

In 339 patients with chronic lymphocytic leukemia the median β2-microglobulin level of 2.4 mg/l has been reported as one of predictors for progression free survival (Letestu et al. 2010) and similar findings were obtained also by Gatto et al. (2003) for β2-microglobulin level of median 2.6 mg/l (range 0.8 – 10.2) in 553 patients with myelodysplastic syndrome.

In our cohort (Table 3) we found 924/1213 (76.3 %) β2-MG <2.0 ng/ml in females and 584/834 (70.0 %) in males, while the prevalence of <3.0 ng/ml appeared negligible.

It should be explained that, first of all, our multipurpose study was predominantly epidemiological, one of our purposes being focused on the distribution of marker levels and their interrelations with organochlorines without any clinical data available on some disease considered specifically related to

certain biomarker. Although the data obtained were submitted back to each of 28 district physicians all appropriatethe suspected or diagnosed cancer in the examined subjects. Thus, from the above presented and discussed clinical findings obtained by others it may be suggested that the cut/off levels of 2.0 ng/ml for CEA, 2.0 IU/ml for AFP and 2.0 µg/ml for beta2-MG we selected and used for the segregation of those possibly threatened by some pathological process, may be considered as acceptable.

References

Bastion Y, Sebban C, Berger F, Felman P, Salles G, Dumontet C, Bryon PA, Coiffier B: Incidence, predictive factors, and outcome of lymphoma transformation in follicular lymphoma patients. J Clin Oncol 15, 1587-1594, 1997.

Bataille R, Durie BG, Grenier J: Serum beta2 microglobulin and survival duration in multiple myeloma: a simple reliable marker for staging. Br J Haematol 55, 439-447, 1983. doi:10.1111/j.1365-2141.1983.tb02158.x

Chen CH, Lin ST, Kuo CL, Nien CK: Clinical significance of elevated alpha-fetoprotein (AFP) in chronic hepatitis C without hepatocellular carcinoma. Hepatogastroenterology 55, 1423-1427, 2009.

Chovancova J, Kočan A, Jursa S: PCDDs, PCDFs and dioxin-like PCBs in food of animal origin (Slovakia). Chemosphere 61, 1305-1311, 2005. doi:10.1016/j.chemosphere.2005.03.057

Gatto S, Ball G, Onida F, Kantarjian HM, Estey EH, Beran M: Contribution of β-2 microglobulin levels to the prognostic stratification of survival in patients with myelodysplastic syndrome (MDS). Blood 102, 1622-1625, 2003. doi:10.1182/blood-2002-10-3264

Greipp PR, Lust JA, O'Fallon M, Katzmann JA, Witzig TE, Kyle RA: Plasma cell labelling index and β-microglobulin predict survival independent of thymidine kinase and C-reactive protein in ultiple myeloma. Blood 81, 3382-3384, 1993.

Hovander L, Linderholm L, Athanasiadou M, Athanassiadis I, Bignert A, Fangstrom B, Kocan A, Petrik J, Trnovec T, Bergman A: Levels of PCBs and their metabolites in the serum of residents of a highly contaminated area in eastern Slovakia, Environ Sci Technol 40, 3696-3703, 2006. doi:10.1021/es0525657

Hue KQ, Kyulo NL, Lim N, Elhazin B, Hillebrand DJ, Bock T: Clinical significance of elevated alpha-fetoprotein (AFP) in patients with chronic hepatitis C, but not hepatocellular carcinoma. Am J Gastroenterol 99, 860-865, 2004. doi:10.1111/j.1572-0241.2004.04152.x

Jiang J, Wu C, Shen Y, Xu B, Zheng X, Li X, Xu N: Clinical Application of Determining Serum AFP- IgM Complexes for Diagnosis of Small Hepatocellular Carcinoma. Anticancer Res. 31, 687- 691, 2011.

Jursa S, Chovancová J, Petrik J, Loksa J: Dioxin-like and non-dioxin-like PCBs in human serum of Slovak population. Chemosphere 64, 686-691, 2006. doi:10.1016/j.chemosphere.2005.10.048

Kantarjian HM, Smith T, Estey E, Polyzos A, O'Brien S, Pierce S, Beran M, Feldman E, Keating MJ: Prognostic significance of elevated serum beta 2-microglobulin levels in adult acute lymphocytic leukemia. Am J Med 93, 599-604, 1992. doi:10.1016/0002-9343(92)90191-D

Kocan A, Petrik J, Drobni B, Chovancovi J: Levels of PCBs and some organochlorine pesticides in the human population of selected areas of the Slovak Republic. Chemosphere 29, 2315-2325, 1994. doi:10.1016/0045-6535(94)90400-6

Kočan A, Petrik J, Jursa S, Chovancova J, Drobna B: Environmental contamination with polychlorinated biphenyls in the area of their former manufacture in Slovakia. Chemosphere 93, 595-560, 2001.

Langer P, Kocan A, Tajtakova M, Petrik J, Chovancova J, Drobna B, Jursa S, Radikova Z, Koska J, Ksinantova L, Huckova M, Imrich R, Wimmerova S, Gasperikova D, Shishiba Y, Trnovec T, Šebokova E, Klimes I: Fish from industrially polluted freshwater as main source of organochlorinated pollutants and increased frequency of thyroid disorders and dysglycemia. Chemosphere 67, S379-S385, 2007a. doi:10.1016/j.chemosphere.2006.05.132

Langer P, Tajtakova M, Kocan A, Petrik J, Koska J, Ksinantova L, Radikova Z, Ukropec J, Imrich R, Huckova M, Chovancova J, Drobna B, Jursa S, Vlcek M, Bergman A, Athanasiadou M, Hovander L, Shishiba Y, Trnovec T, Šebokova E, Klimes I: Thyroid ultrasound volume, structure and function after long-term high exposure of large population to polychlorinated biphenyls, pesticides and dioxin. Chemosphere 69, 118-127, 2007b. doi:10.1016/j.chemosphere.2007.04.039

Langer P, Kocan A, Tajtakova M, Petrik J, Koska J, Ksinantova L, Radikova Z, Ukropec J, Imrich R, Huckova M, Chovancova J, Drobna B, Jursa S, Vlcek M, Bergman A, Athanasiadou M, Hovander L, Shishiba Y, Trnovec T, Šebokova E, Klimes I: Possible effects of persistent organochlorinated pollutants cocktail on thyroid hormone levels and pituitary-thyroid interrelations. Chemosphere 70, 110-118, 2007b. doi:10.1016/j.chemosphere.2007.06.046

Letestu R, Vincent Levy V, Eclache V et al.: Prognosis of Binet stage A chronic lymphocytic leukemia patients: the strength of routine parameters. Blood 116, 4588-4590, 2100.

Linderholm L, Park JS, Kocan A, Trnovec T, Athanasiadou M, Bergman K, Hertz-Picciotto I: Maternal and cord serum exposure to PCB and DDE methyl sulfone metabolites in eastern Slovakia, Chemosphere 69, 403-410, 2007. doi:10.1016/j.chemosphere.2007.04.081

Mailey B, Artinyan A, Khalili J, Denitz J, Sanchez-Luege N, Sun CL, Bhatia S, Nissen N, Colquhoun SD, Kim J: Evaluation of absolute serum alpha-fetoprotein levels in liver transplant for hepatocellular cancer. Arch Surg 146, 26-33, 2011. doi:10.1001/archsurg.2010.295

Nielsen HJ, Brünner N, Jorgensen LM, Olsen J, Rahr HB, Thygesen K, Hoyer U, Laurberg S, Stieber P, Blankenstein MA, Davis G, Dowell BL, Christensen IJ: Plasma TIMP-1 and CEA in detection of primary colorectal cancer: a prospective, population based study of 2509 high-risk individuals. Scand J Gastroenterol 46, 60 – 69, 2011.

Park J-S, Linderholm L, Charles JM, Athanasiadou M, Petrik J., Kocan A.: Polychlorinated biphenyls and their hydroxylated metabolites (OH-PCBs) in pregnant women from eastern Slovakia. Environ Health Perspect 2007; 115: 20-7. doi:10.1289/ehp.8913

Park J-S, Bergman A, Linderholm L: Placental transfer of polychlorinated biphenyls, their hydroxylated metabolites and pentachlorophenol in pregnant women from eastern Slovakia. Chemosphere 2008; 70: 1676-1684. doi:10.1016/j.chemosphere.2007.07.049

Petrik J, Drobna B, Pavuk M, Jursa S, Wimmerova S, Chovancova J: Serum PCBs and organochlorine pesticides in Slovakia: Age, gender, and residence as determinants of organochlorine concentrations. Chemosphere 18, 410-418, 2006. doi:10.1016/j.chemosphere.2006.02.002

Shin WY, Suh KS, Kim T, Jeon YM, Yi NJ, Kee KU: Could patients who underwent hepatic resection due to hepatocellular carcinoma with high alpha-fetoprotein be monitored for recurrence by alpha-fetoprotein level? Korean J. Hepatol. 16: 168-175, 2010. doi:10.3350/kjhep.2010.16.2.168

Tai WC, Hu TH, Wang JH, Hung CH, Lu SN, Changchien CS, Lee CM: Clinical implications of alpha-fetoprotein in chronic hepatitis C. J. Formos. Med. Assoc. 108, 210-218, 2009. doi:10.1016/S0929-6646(09)60054-1

Tan E, Nikos Gouvas N, R. John Nicholls RJ, Ziprin P, Xynos E, Tekkis PP: Diagnostic precision of carcinoembryonic antigen in the detection of recurrence of colorectal cancer. Surgical Oncology 18, 15-24, 2009. doi:10.1016/j.suronc.2008.05.008

Xu L, Wang Y, Wu W, Yan H, Gao XD, Yu Q, Shen ZX, Mi JQ: Levels of beta-2 microglobulin can be elevated in multiple myeloma and lymphoma, though in these cases primary amyloidosis (amyloid light chain) and secondary amyloidosis (Amyloid associated protein) are more common. Zhonghua Yi Xue Za Zhi. 13, 972-977, 2010.

Zenz T, Fröhling S, Mertens D, Döhner H, Stilgenbauer S: Moving from prognostic to predictive factors in chronic lymphocytic leukaemia (CLL). Best Pract Res Clin Haematol 23, 71-84, 2010 doi:10.1016/j.beha.2009.12.003