Abstract of Theses from the Nordic Countries

Abstract of Nordic theses on oncologic subjects are published under this heading. The full theses are as a rule published by the universities or as supplements to different journals. They can usually be obtained after contact with the author.

Cytotoxicity and DNA damage in human normal and neoplastic cells exposed to bifunctional DNA-reactive cytostatic agents

J. HANSSON

Department of General Oncology, Radiumhemmet, Karolinska Hospital, S-104 01 Stockholm, Sweden

The effects of bifunctional DNA-reactive cytostatic drugs on human melanoma cells and normal human phytohaemagglutinin (PHA)-stimulated lymphocytes were compared. The cytotoxicity of melphalan and cis-diamminedichloroplatinum (II) (cis-DDP), as measured by the inhibition of ^{3}H-thymidine incorporation, was lower in melanoma cells as compared to lymphocytes. Measurements of cellular incorporation of ^{3}H-melphalan, of cellular content of free melphalan and cellular platinum uptake indicated that the reduced cytotoxicity in melanoma cells was not due to a reduced drug uptake. The cellular content of glutathione was higher in melanoma cells as compared to lymphocytes, which is likely to contribute to the reduced toxicity of melphalan. The reduced cytotoxicity of melphalan and cis-DDP in melanoma cells was accompanied by lower levels of DNA cross-linking, as measured by alkaline elution.

The cytotoxicity of melphalan and nitrogen mustard (HN2) in melanoma cells was shown to be related to both the induction of DNA interstrand cross-links and the rate of removal of these lesions.

Lowering of cellular glutathione content by treatment with buthionine sulfoximine (BSO) sensitized melanoma cells to HN2 and melphalan, while the effects on cis-DDP- and bischloroethylnitrosourea-induced cytotoxicity were much smaller. The potentiation of drug-induced cytotoxicity was correlated to similar enhancement of DNA cross-linking. No effect of BSO on the rate of repair of HN2-induced DNA cross-links was observed.

The cytotoxicity of m-L-sarcolysin in PHA-stimulated lymphocytes was much higher than that of melphalan, with intermediate cytotoxicity following exposure to peptide-bound m-L-sarcolysin (peptichemio). The differences in cytotoxicity were parallelled by similar differences in drug-induced DNA cross-linking. Measurements of cellular content of free m-L-sarcolysin and melphalan indicate that the difference in cytotoxicity is likely to be caused by a differential uptake of these drugs.

February, 1987

Karyometric investigations on urinary bladder carcinoma, including histotechnological studies with special reference to nuclear morphology

K. G. HELANDER

Department of Pathology, Umeå University, S-901 87 Umeå, Sweden

The aim of the investigation was to characterize the structure of the cell nuclei in urinary bladder carcinoma. Special studies were devoted to the influence of different steps in the processing for microscopy on nuclear structure.

Dehydration in general causes shrinkage of tissues. In this part of the thesis the size of the cell nuclei was recorded after dehydration in ethanol or acetone. Furthermore, the rate of water extraction was studied. The largest nuclei, indicating the least shrinkage, were observed in tissues dehydrated in rising concentrations of ethanol. Dehydration was somewhat faster in acetone than in ethanol, and shaking of the vials accelerated the dehydration considerably, but could cause mechanical damage to tissues. Dehydration was fast and gentle with intermittent shaking.

Sectioning results in compression of embedded tissues. The degree of compression was studied in paraffin and plastic embedded tissues after microtomy when glass knives of different edge angles were employed. There was a good correlation between edge angle and the degree of section compression. Compression was less marked in plastic than in paraffin sections. In the paraffin sections nuclear compression averaged 11% and in the plastic sections 4%.

Using interference microscopy the surface topography of paraffin and plastic sections was studied. Plastic sections were more even than paraffin sections, in which the cell nuclei often gave rise to marked thickness variations.

Based on these findings morphometry was carried out by light microscopy in two series of biopsies from urinary bladder epithelium, including material from urothelial carcinomas. The biopsies were fixed, dehydrated in rising concentrations of ethanol, embedded in glycol methacrylate and cut in 1.5 μm thick sections using glass knives. In one of these series flow cytometry was carried out as well on parallel biopsy material. These two studies showed that the higher grades of malignancy were characterized by large nuclei and high nuclear volume density; however, in undifferentiated carcinoma the nuclei were small. Flow cytometry revealed an aneuploid DNA pattern in all grade III tumours and in about 1/3 of the grade II tumours.

An additional study was carried out on a larger material of cell suspensions from urinary bladder biopsies. Nuclear size was measured by morphometry and DNA content by flow cytometry. All grade III tumours and 2/3 of the grade II tumours were aneuploid. In general, these cases displayed large nuclei. Two thirds of the diploid grade II tumours could be distinguished from normal epithelium by their large nuclei.

April, 1986

Radiological diagnosing of liver tumours
Evaluation of angiography and intravenous and intra-arterially enhanced computed tomography

C. LUNDSTEDT

Department of Diagnostic Radiology, University Hospital, Lund University, S-221 85 Lund, Sweden

Sixty patients treated with an intra-arterial cytostatic drug for metastases from colo-rectal carcinoma were evaluated with angiography to determine prognostic parameters. The extent of tumour in the liver and an unchanged or diminished tumour volume following treatment, as demonstrated with angiography, were associated with significant prolongation of survival. Patients who developed occlusion of the hepatic artery or of branches of the portal vein, also survived longer. 189 patients examined with angiography, 161 with computed tomography (CT), 95 with computed tomographic arteriography (CTA) and 71 with ultrasound (US) were subjected to liver evaluation at laparotomy consisting of inspection and palpation. The result of this surgical liver evaluation was for the purpose of the study regarded as completely accurate and was used to assess the accuracy of the different radiological methods. The location of tumour in the liver lobes or segments was analysed, with a separate evaluation of the right and left liver lobes. The rate of detection of individual tumour nodules was also determined.

Angiography detected 55% of liver areas affected by tumour

and 47% of individual tumour nodules. CT detected 83% of liver lobes or segments containing tumour, and 70% of the tumour nodules. US detected 69% of the portions of liver holding tumour, and also 69% of the tumour nodules. CTA detected 85% of tumorous areas and 74% of separate tumour nodules.

Some lesions detected with CT were not seen with CTA and vice versa. More false-positive results were recorded with CTA than with CT using intravenous contrast enhancement.

February, 1987

Assessment of exposure to tobacco smoke.
Application of sister chromatid exchange as the cytogenetic endpoint

K. HUSGAFVEL-PURSIAINEN

Institute of Occupational Health, University of Helsinki, Helsinki, Finland

A combination of various biochemical and biological indicators of exposure was applied in assessing tobacco smoke exposure of active and passive smokers under both experimental and natural exposure conditions. Sister chromatid exchange (SCE) in peripheral blood lymphocytes was utilized as the principal biological endpoint: SCEs are intrachromosomal exchange events analysed under the light microscope and, as far as is presently known, they are sensitive indicators of damage to cellular DNA. The frequency of SCEs in lymphocytes was found to be significantly higher among smokers (almost exclusively cigarette smokers) as compared with non-smokers taken as a group. The SCE frequencies of smokers showed a dependence on the amount and duration of smoking. However, non-smokers exposed to environmental tobacco smoke (ETS) had SCE values comparable to those of the non-exposed non-smokers. The biochemical measures of absorption of tobacco smoke constituents used in the study included carboxyhaemoglobin (COHb) for carbon monoxide exposure, plasma thiocyanate (P-SCN) for hydrogen cyanide exposure, and plasma and urinary cotinine (P-cot, U-cot) for nicotine exposure. Of these measures, only cotinine as the major metabolite of nicotine is specific for tobacco. The values of all these parameters were significantly elevated indicating exposure of smokers. Non-smokers with work-related passive exposure were observed to have increased levels of plasma thiocyanate and plasma and urinary cotinine. However, passive exposure of non-smokers under controlled experimental conditions caused elevation of urinary cotinine values only. The results of the present study support the use of cotinine as the most suitable marker for intake of tobacco smoke constituents. Mutagenic activity in urine samples studied with the bacterial fluctuation test was significantly higher in smokers than in non-smokers. In both experimental and natural situations of environmental tobacco smoke exposure, passive smokers showed a trend of slightly increased urinary mutagenicity. The ETS exposure of non-smokers was also characterized by collecting samples of airborne particulate matter from restaurants. The extracted samples were analysed for polynuclear aromatic hydrocarbones (PAHs) as well as for the induction of mutations in the Salmonella/*microsome* assay and the induction of SCEs in the Chinese ovary cells. Both of the assays used revealed genotoxic activity in the ETS samples.

March, 1987

Effects of irradiation on growing bones

H. ENGSTRÖM

Department of Oral Radiology, University of Göteborg, S-400 33 Göteborg, Sweden

The effects of irradiation on various tissues have been studied extensively. Nonetheless, the metabolism in growing bones has not been evaluated in a systematic way after moderate doses of irradiation. It was found that scattered radiation, that reaches the oral region during radiotherapy of malignancies outside the oral region, causes absorbed doses within the range of 0.2–20 Gy, while absorbed doses from radiography in orthodontics were only 30–40 mGy. Bone formation in the metaphyseal area of rat tibia *in vivo* after irradiation with 0.5–8 Gy was determined by a tetracycline labelling method. Five and 8 Gy induced a significant growth retardation. This was detectable already after 36 hours and was maximal 7–14 days after irradiation. Between 14 and 30 days following irradiation growth was normalized. Alkaline phosphatase (ALP) activity in bone was evaluated biochemically and decreased one day after irradiation with 0.5–8 Gy. This was followed by a gradual increase in ALP activity and a return to normal values 30 days after irradiation. Histochemical studies of the rat tibias included evaluation of ALP, acid phosphatase, $NADH_2$-diaphorase and Glucose-6 phosphate dehydrogenase. A decrease in ALP activity one day after irradiation was observed with 5, 8, and 10 Gy. Acid phosphatase and the two oxidative enzymes were increased in activity during the entire 7-day experimental period, reflecting an altered metabolism. Normal activities of all the studied enzymes were observed 30 days after irradiation. Results from suture area and synchondrosis area as evaluated by histochemistry and a cephalometric radiographic method showed that early transient metabolic changes occurred in the craniofacial growth sites after irradiation with 5 and 8 Gy. The morphological changes observed in anatomical regions within the irradiated field (neurocranium) persisted in contrast to the changes in the viscerocranium that were normalized at the end of the experimental period. An *in vitro* system was used to examine the effects of irradiation on certain aspects of bone growth. Mice calvaria were irradiated *in vitro* with 2 or 10 Gy. A different response in suture and bone was found 3 hours to 4 days after irradiation. Bone was affected by 2 Gy, but not the suture. Thus, the suture seems to be an area with more radioresistant fibroblast-like cells than the cortical bone, which indicates a difference in radiosensitivity of the cells in these two growth sites.

The *conclusions* from the present thesis are that irradiation with 2–10 Gy of bone both in organ culture and in experimental animals induces metabolic and morphologic changes which were detected early and were transient.

March, 1987

Establishment, characterization and sensitivity studies of small cell lung carcinoma in vitro and transplanted to athymic mice

S. A. ENGELHOLM

Department of Medicine, University of Copenhagen, Copenhagen, Denmark

Human small cell carcinoma of the lung (SCCL) was established as *in vitro* cell lines and transplanted into athymic (nude) mice.

A new method to establish fibroblast-free cell lines was developed. The tumors in patients and in the model systems were characterized by series of biological properties. The stability of some of the properties were checked *in vitro* as well as in nude mice over a period of one to five years. It was demonstrated that SCCL preserve important characteristics in the model systems. The heterogeneity of SCCL was reflected *in vitro* as well as in

nude mice and diversity in cellular DNA content, cell cycle distribution, and macroscopic growth was observed even among subpopulations isolated from the same tumor. Genetic instability was observed in some tumors *in vitro* as well as in nude mice.

The heterotransplanted tumors were used in chemotherapy experiments. Pronounced heterogeneity in sensitivity was found, although the tumors were established from previously untreated patients. A correlation between chemosensitivity and specific cell cycle perturbations was shown.

The tumors were used for demonstration of a new principle for estimating chemosensitivity *in vitro*. Flow cytometry was used to measure drug-induced cell cycle perturbations after *in vitro* incubation with the drug. Ehrlich ascites tumors were used to development of the method. A method to disaggregate solid tumors to a single cell suspension was developed, and hereafter the flow cytometry assay was performed on disaggregated human heterotransplanted tumors.

The results indicate that the method is applicable as a sensitivity test in general, encompassing screening of new drugs.

August, 1987

Epidemiology in planning for health with special reference to regional epidemiology and the use of health registers

M. Rosén

Department of Social Medicine, University of Umeå, Umeå, Sweden

The overall aim of this thesis is to assess the potential for using epidemiological data from available registers as a tool for planning. Specific aims are to assess the validity of particular data files and to illustrate how ecological analyses can be utilized as a basis for health planning. A strategy for planning is also suggested.

The potential for prevention as revealed by epidemiological research and deficiencies in the planning methods used in Sweden were the main points of departure for the project 'Health Problems in a County—a Basis for Health Planning'. This collaborative project between 1978 and 1984 strongly related to the planning activities in the Västerbotten County Council forms the basis for this thesis.

Numerous registers are available in Sweden concerning health status and utilization of care, and their determinants. Data from these registers are usually available for small geographical areas. Information is, however, limited concerning data on ambulatory care, lifestyle, functional impairment etc. The review of data quality in official registers revealed several deficiencies which can partly be overcome. The assessed registers can then display quite accurate data on an ecological level.

Regional variations in mortality are a planning base of major interest in this work and the validity of presented data is crucial. Guidelines for validating these regional variations are suggested. Ecological analyses can be a valuable tool in generating hypotheses and directing measures according to local needs. Empirical results from three ecological studies illustrate the potentials and limitations of this approach. These ecological studies show, for example, that cardiovascular disease (CVD), diabetes, and stomach cancer are more prevalent in the northern parts of Sweden, while colorectal cancer is more common in the south of Sweden. Several of suspected CVD risk factors (hypertension, saturated fatty acid, salt, diabetes, obesity, coffee, soft water, and unemployment) cluster in the north of Sweden, smoking being the only exception. Furthermore, a high negative correlation was found between fibre intake and colorectal cancer. Similar associations with colorectal cancer are also indicated for milk consumption or calcium intake. This thesis discusses the potentials of using epidemiological data in planning as a way of identifying and monitoring health problems, allocating resources, and assessing different measures. The questions of equal access to care and health equality in Sweden are reviewed.

A strategy in planning for health is also suggested and tested in the County of Västerbotten. The results indicate that certain prerequisites can contribute to successful plan implementation. These prerequisites include the need to compile, analyse, and disseminate community diagnoses and to create opportunities for physicians and other health personnel to analyse local, epidemiological data and discuss the appropriateness of different intervention strategies. Several projects have been initiated in Sweden as a result of presenting local, comparative data. The case of a community-based intervention against CVD in Norsjö, Västerbotten, is presented, starting with identifying the magnitude of CVD, up to the initial stages of implementation.

May, 1987

Organization, functions and effectiveness of follow-up for breast cancer patients

K. Holli

Institute of Public Health, Tampere University Central Hospital, Tampere, Finland

Breast cancer is the most common form of cancer among Finnish women. Due to the increase in the risk of cancer, to the aging of population and to an improvement in the survival rate, the number of cases is increasing. In 1980 the number of prevalent cases was 12610 and according to predictions for the year 2000 it will be 44000. Prevalence is the indicator of needs of services for the follow-up of cancer patients.

Planning of the follow-up activities assumes as a first step a situation analysis of both the organisation and functions of the present follow-up and after-care of the breast cancer patients. This study describes the 5-year follow-up for breast cancer patients residing in Tampere University Central Hospital area and diagnosed in 1977–1980. There were 551 patients with 8248 visits during the first 5 years of follow-up.

Most of the women (75%) found a lump in the breast or other signs and symptoms by themselves. Primary health centers and private practitioners diagnosed the suspicious lesion usually by clinical examination only. Diagnostic confirmation was made and primary course of treatment was given by specialised surgery either at the university clinic, at a regional hospital or by a specialist at the primary health center with surgical facilities. Most of the follow-up visits (72%) occurred at the radiotherapy clinic of the university central hospital. Because the relapses and terminal cases were also as a rule treated at the radiotherapy clinic, the use of services for follow-up was mainly at the central hospital level, especially at the radiotherapy clinic.

There is a general recommendation in Finland by the National Board of Health and an agreement between the specialised cancer clinics to have a follow-up visit every 3 months during the first year of follow-up and every 4 months during the second year. After the second year of follow-up the visits should be repeated every 6 months until the end of the fifth year of follow-up and annual follow-up visits are recommended thereafter. According to the recommendation, the expected number of visits was 4873 whereas the observed number was 8248. The visits consisted mainly of diagnostic activities directed at the detection of relapses and treatment procedures aiming at a cure of the cancer. There were an average of 3.4 radiographic chest examinations and 3.9 laboratory examinations per patient-year. The frequency of diagnostic tests and nonpalliative treatment increased with the progression of the disease and was most inten-

sive during the terminal stage less than one week before death. The focus on the cancer preventing treatment and cure aimed at improving or maintaining the mental, social and physical well-being and quality of life of the patients. Rather, the disease oriented diagnostic and curative activities probably made the quality of life worse.

The mean number of visits per patient during the first 5 years of follow-up was 16. There were an average of 11 different physicians or surgeons for these 16 visits. Therefore most patients met at every follow-up visit a new doctor. This implies that a doctor–patient relationship was an unrealistic goal due to administrative and other causes or such a relationship was not regarded important.

Because the study was a nonexperimental retrospective one, only indirect evidence emerged on the effects of follow-up on the relapse and outcome of the disease, on the quality of life in terms of mental and physical well-being, and on the social competence. No direct evidence supported the hypothesis that frequent follow-up visits at university central hospital improved the outcome of the disease in terms of survival. Because the visit consisted of diagnostic and therapeutic activities aimed at the disease, the outcome cannot be a general improvement of the physical, mental or social well-being of the patient. Rather, the reverse may be true. Even if there are some indirect effects of the follow-up it is likely that the effectiveness and efficiency would be improved if direct activities aimed at the well-being of the patient were used.

The recommendations on the frequency of the follow-up visits are not based on empirical observations. They seem to be rather remote from the actual practice. It is likely that removal of the follow-up from oncological clinics to closer to the place of residence and closer to the primary health services of the patient for patients without recurrence of the disease would not materially affect the survival. Such a change may improve the quality of physical, mental and social life of the patient and allow the frequency of the follow-up visits to get better adjusted according to the needs of the patients.

August, 1987

Recurrent intra-abdominal cancer

J. MÄKELÄ

Department of Surgery, University of Oulu, Oulu, Finland

A retrospective analysis of 178 consecutive patients with recurrent intra-abdominal cancer was undertaken to evaluate the rationale and efficacy of surgical treatment. All 178 patients required operative treatment for gastrointestinal problems caused by recurrent cancer. In addition, the clinical course of 424 patients with primary intra-abdominal cancer was evaluated.

The overall operative mortality and morbidity after operations for recurrent cancer were 20% and 46% respectively, the mean and median survivals 13±20 and 7 months, and the cumulative 5-year survival 5%. Fifty-seven (32%) of the 178 patients survived for over one year and 9 (5%) for over 5 years. The mean and median relief of preoperative symptoms were 8±18 months and 2 months.

In patients with locoregional recurrences, the mean and median survivals (34±29 and 35 months) and mean and median relief of symptoms (22±18 and 10 months) after resection were significantly longer than after other procedures, as was the cumulative 5-year survival (8%). In patients with distant recurrences, resection of the intra-abdominal tumour also gave better mean and median survivals (14±21 and 7 months) and relief of symptoms (10±24 and 5 months) than other treatment alternatives. Resection did not significantly raise mortality or morbidity, in patients with locoregional or distant recurrences. The mortality and morbidity rates after operations performed as emergencies were high, 41% and 69% respectively, and after operations performed electively 14% and 39%. Patients with recurrent gynecological cancer had a slightly shorter survival time and symptom relief than other patient groups.

The recurrence rate after all resections was 61% and after radical resections 46%. The corresponding recurrence rates in gastric cancer were 69% and 50%, in colorectal cancer 50% and 36% and in pancreatic cancer 83% and 79%. 39% of the patients with recurrent intra-abdominal cancer were reoperated.

When the results of surgical treatment were compared with those treated nonoperatively, only resection appeared to clearly prolong survival. On the basis of these results it is concluded that careful preoperative evaluation of the extent of cancer growth and that after this a resective procedure is recommendable, when possible, as resection can most often offer comfortable palliation and prolonged survival.

August, 1987

Human milk fat globule membrane antigens as diagnostic markers for ovarian carcinoma

P. ASHORN

Department of Biomedical Sciences, University of Tampere, Tampere, Finland

Fat globules in human milk are surrounded by a bi-layer membrane derived from the plasma membrane of secreting mammary epithelial cells. The aim of this study was to use this readily obtainable source of plasma membrane proteins as immunogen to obtain antibodies reacting with differentiation antigens in normal and especially malignant epithelial tissue. Fifteen monoclonal cells lines producing antibodies with different specificities were generated using the standard murine hybridoma technique. All these antibodies reacted with antigens expressed by malignant cells of ovarian or mammary origin.

Monoclonal HMFG antibodies were used to characterize the corresponding antigens by immunochemical methods. HMFG antigens were shown to be large and complex glycoproteins, especially rich in galactose and N-acetylgalactoseamine. Using fractionation with SDS polyacrylamide gel, a glycoprotein with a molecular weight of 42–57 kDa was isolated. This antigen was exclusively stained in immunoblotting with monoclonal antibody HMFG IIID5 that has previously been shown to detect antigenic material in estrogen receptor positive but not negative breast tumors. The 42–57 kDa antigen was shown to contain large amounts of galactose but only limited amounts of other sugar residues.

Since monoclonal HMFG antibodies detect tumour associated antigens in ovarian carcinoma, these antibodies were used in immunohistochemistry for differential diagnosis of these tumors. Serous, mucinous, endometrioid and mesonephroid carcinomas all showed different staining patterns, when an immunohistochemical panel consisting of HMFG antibodies HMFG IIIC12 and SM IF3 and peanut agglutinin lectin were used. The results suggest that such a staining method can be used to improve the differential diagnosis of poorly differentiated ovarian tumors.

A sandwich ELISA for circulating HMFG was developed using the monoclonal HMFG IIIC12 antibody and a polyclonal antiserum. All healthy medical students tested had a serum value below 400 U/ml, whereas values above this cut-off limit were observed in 50% of ovarian cancer patients. A correlation was found between a rising antigen level and enlarging tumour mass. As to control material, none in a limited number of the patients with inflammatory disease, 35% of patients with advanced breast cancer and only 3% of the patients with other malignancies than ovarian or breast cancer showed elevated serum HMFG levels.

To find out whether monoclonal HMFG antibodies also in vivo bind to malignant cells, a small dose of radiolabelled antibodies were administered intraperitoneally in patients suffering from widespread ovarian carcinoma. Anteroposterior scanning and computed tomography images were taken 5 and 21 h after the injection to show the localization of the antibodies. A good correlation between the accumulation of radioactivity and the localization of carcinoma metastases observed at laparotomy was found. A larger dose of radiolabelled antibody was later administered intraperitoneally to the same patient to attempt antibody-guided radiotherapy but only a transient relief in ascites formation could be demonstrated.

August, 1987

Diagnostic procedures and biomarkers in the detection of CNS metastases from small cell lung cancer

A. G. PEDERSEN

Department of Medicine, University of Copenhagen, Copenhagen, Denmark

In patients with SCLC the diagnosing of CNS metastases remains a difficult task. Considerable progress has been made with the application of CT-scannings. A number of patients, however, remains undiagnosed by CT-scanning despite strong neurologic evidence for the presence of brain metastases. Furthermore a significant number of patients do not have their metastases detected while alive. A very limited number of patients (5%), on the other hand, can, although asymptomatic, have CNS metastases diagnosed by CT-scan. In the diagnosis of meningeal carcinomatosis or spinal metastases CT-scanning is relevant only in selected cases. Of newer imaging techniques nuclear magnetic resonance (NMR) scanning seems promising. At present, however, no larger series of patients with SCLC and possible brain metastases have been published. This technique also offers the possibility of looking at metabolic changes in metastases and perhaps also of tracing cytostatic compounds when given to patients with metastases.

The neurologic examination is an important complimentary investigation both with regard to brain metastases, meningeal carcinomatosis and spinal cord compression. Although it is too inaccurate to serve as a screening procedure it should not be abandoned because either type of CNS metastases at times only will be detected by the neurologist.

Myelography remains a very important diagnostic tool in the diagnosis and delineation of spinal cord compression. In addition newer water soluble contrast media seem to improve visualization of spinal meningeal seedings in a substantial number of cases. Accordingly myelography also in this respect becomes a supplementary diagnostic tool to cytologic examination of CSF.

CSF cytology analysis remains the most important diagnostic means in detecting meningeal carcinomatosis. Due to a substantial number of false negative conclusions, however, the determination of biomarkers in CSF may deserve increased interest.

The original hopes for a high specificity in the CSF analysis of biomarkers produced by SCLC has only partially been fulfilled. The 2 markers first investigated AVP and ACTH gave disappointing results. This was accounted for partially by other competing causes resulting in increased AVP but mostly due to the lack of production of these biomarkers in a substantial proportion of tumors.

The most rewarding biomarkers investigated as yet, have been CK-BB and bombesin which have been found specifically to be elevated in patients with meningeal carcinomatosis. The lack of increase in concentration of these markers in patients with parenchymal metastases may be due to rapid degradation in brain tissue, since both of these substances normally are very abundant in brain tissue. With the production of monoclonal bombesin antibody a new means of diagnosing and perhaps treatment of meningeal carcinomatosis seems possible.

NSE and Calcitonin were the markers most closely associated with brain metastases. Neither of them, however, offers any diagnostic potential due to the relatively frequent false negative outcome when compared to CT-scanning. Development of newer assays with a higher specificity may conceivably improve the diagnostic accuracy of all these markers. Furthermore antibodies 'seeing' different parts of a marker molecule are being developed. Some of these may detect aminoacid sequences within the peptide molecule, which are more consistently produced by SCLC cells. So although only limited clinical progress has been made by applying biomarkers in the detection of CNS metastases, this may be an area likely to benefit from the use of biomarkers in the future.

September, 1987

Cancer family syndrome. Studies on the hereditary nonpolypous colorectal carcinoma syndrome

J.-P. MECKLIN

Second Department of Surgery, Helsinki University Central Hospital, Helsinki, Finland

The cancer family syndrome (CFS) is a hereditary nonpolyposis colorectal carcinoma (CRC) syndrome, for which dominantly inherited tendency to CRCs and to certain other adenocarcinomas, at a young age, has been suggested. However, the frequency of CFS has not been available so that the clinical significance of this disorder has remained unknown.

The aim of the present study was directed to assessing the frequency of CFS in the Finnish population and elucidating the questions of identification and clinical characteristics of this heritable syndrome. It was also attempted to evaluate the therapeutical implications of a diagnosis of CFS in the case of an individual patient and the rationale for screening of colorectal neoplasms in asymptomatic descendants of the affected CFS-patients. In the present investigation the frequency of hereditary CRC was evaluated in a regional sample of all CRC patients (n=468) in a 10-year period. The method was to study the causes of death of the parents of all patients under 70 years of age (n=241; 51%) using population registers. If one of the parents had died of cancer, the family was studied further. This information was not obtained for the patients over 70 years of age (n=227; 49%) for two reasons. Firstly, it was known that hereditary CRC-syndromes arise very seldom in old patients. Secondly, the death certificates of the parents of these patients dating back to the beginning of this century were considered to be uninformative in many instances. Four % (18 patients) of all CRC patients proved to belong to CFS families and another 2% (8 patients) were probable CFS cases. One patient (0.2%) with familial polyposis coli (FPC) was found in this series. Thus, CFS was 20-fold more common than FPC. The CFS patients were young (mean 41 years), and on the basis of the present study about one third or even more of the CRC patients under 50 years of age belong to CFS families. The observed frequency of CFS (4–6% of all CRC patients) is perhaps an underestimate, because it was possible to identify only those CFS families with inherited cancer cases in two or more generations. However, solitary CFS cases must also occur, but they cannot be revealed until a specific biomarker for CFS is available.

The main genetic, histopathological and clinical characteristics of CFS were elucidated in a series of 22 kindreds (200 affected patients), collected essentially independently of the epidemiological study. Autosomal dominant transmission of the syndrome

could already be suggested on the basis of the pedigrees. This was further confirmed by counting the number of affected patients in each generation. The observed figures for colorectal, endometrial and undefined intra-abdominal carcinomas were 58% in the 2nd, 25% in the 3rd and 11% in the 4th generation being compatible with the expected figures when autosomal dominant inheritance was assumed (50, 25 and 12.5% respectively). The cumulative risk of CRC in the descendants at risk showed a gradual increase in occurrence from 19 years, reaching 50% at the age of 69 years. This also supports the dominant transmission of the syndrome. The risk of cancer in CFS seems to occur between 20 and 65 years of age (peak incidence between 30 and 49 years of age). Healthy family members over 69 years of age did not show an abnormal risk of cancer.

The sex ratio of all family members in the present CFS kindreds was 1:0.85, and it was the same in those affected by carcinoma. However, the sex ratio in those affected by CRC was 1:0.6, but this difference disappeared when the female patients with endometrial carcinoma were included.

The main clinical characteristic in addition to the young age of the carcinoma patients, was the high frequency of multiple malignancies (25%). CRC was also the most common carcinoma as a metachronous tumour. In 35% of the patients surviving for at least a year after discovery of a primary colonic cancer a new metachronous CRC was diagnosed, and the cumulative risk of subsequent CRC was 3.2 to 5.4% per year. Thus, abdominal colectomy with ileorectostomy and life-long follow-up of the remaining colon should be recommended for the CFS-patients with CRC, but the possibility of extracolonic malignancies should be kept in mind. Despite the many typical characteristics of CRC-patients in CFS-kindreds the identification of a single CFS-patient with no familial background is impossible until the other relatives become affected.

The high frequency of associated colorectal adenomas (19%) supported the role of the adenoma–carcinoma sequence even in CFS. The histological aggressiveness of the adenomas (significantly increased proportion of villous features and moderate and severe dysplasia) in CFS patients might signify an accelerated advancement of this phenomenon in CFS. Also mucinous histological features of CRCs associated with CFS in the present study (39 vs. 20%). Although this characteristic is not specific for CFS, it might serve as one sign in identification of the syndrome.

The first screening visit by colonoscopy or by colography and sigmoidoscopy yielded a colonic neoplasm in 9% of the asymptomatic family members at risk (CRC in 2 and colonic adenoma in 10 subjects). This is 20-fold more than screening for CRC in the general population of corresponding age. However, the screening will be continued and the final conclusion about its benefit cannot be drawn before all subjects have passed through the risk age.

As a general conclusion the present study revealed that CFS families are more common than earlier believed and they constitute a risk group of CRC with unquestionable aetiological significance. Thus, a study of the family history for every new patient with CRC is recommended. In verified CFS cases with CRC, abdominal colectomy with ileorectostomy and life-long follow-up of the remaining colon should be recommended. The screening of CRC is also much more effective and better justified in CFS-families than in the general population, but still involves problems concerning the organization and long-term evaluation of the benefits of such screening programs.

September, 1987

C-myc activation by chromosomal translocation in spontaneously arising rat immunocytomas

W. S. PEAR

Department of Tumor Biology, Karolinska Institute, S-10401 Stockholm, Sweden

The cytogenetic and molecular studies described in this thesis were undertaken in order to understand the underlying mechanisms and consequences of the consistent chromosomal translocation identified in spontaneously arising immunocytomas of the Lou/Wsl strain of rats. Karyotypic analysis of 16 rat immunocytomas showed that all 16 tumors contain a chromosome translocation between chromosomes 6 and 7. Through the use of rat/mouse somatic cell hybrids, the immunoglobulin heavy chain locus was localized to chromosome 6 and the c-myc cellular oncogene to chromosome 7. Restriction enzyme analysis has shown that the c-myc is rearranged within a 35 kilobase region surrounding the gene in 12 of 14 tumors. In 10 of these tumors, the site of c-myc rearrangement occurred within a 1.5 kb region located upstreams of exon 1. In contrast to sporadic Burkitt's lymphoma and mouse plasmacytoma, only 1 tumor contained the c-myc breakpoint in intron 1.

Cloning of the rearranged c-myc genes from 4 tumors showed that the c-myc recombined with immunoglobulin heavy chain sequences in a head-to-head transcriptional orientation. This finding shows that 3 different tumors (rat immunocytoma, mouse plasmacytoma, and Burkitt's lymphoma) arising in 3 species (rat, mouse, and human) juxtapose identical genetic loci (c-myc, immunoglobulin) by chromosomal translocation. These three tumors represent at least two different stages of B-cell maturation, and the modes of induction and natural histories of the tumors are very different. The consequence of the translocation in all three tumors is activation of the c-myc locus. The remarkable consistency of c-myc activation by chromosomal translocation in these three tumors suggests that this must be a rate-limiting step in the pathogenesis of these tumors.

Analysis of the sequences juxtaposed to the c-myc showed that IgH switch regions are the targets in at least 5 tumors, and that there is a strong correlation between the secreted immunoglobulin and c-myc target. This suggests that mistakes in immunoglobulin assembly contribute to the illegitimate recombination between the Ig and c-myc loci. This concept is strengthened by the study of two tumors, in which the c-myc recombined with sequences which were distinct from the immunoglobulin switch regions. In both of these tumors, the sequences at the breakpoints shared limited homology with Ig switch regions.

In two tumors, c-myc rearrangement occurred as the result of multiple events. Despite the complex rearrangements in these tumors, the c-myc was juxtaposed with immunoglobulin sequences. This emphasizes the selectivity of translocation of the c-myc into the immunoglobulin locus in the generation of rat immunocytoma, mouse plasmacytoma, and Burkitt's lymphoma.

September, 1987

Uracil-DNA glycosylase activity in human benign and malignant hematopoiesis

P. KOISTINEN

Department of Clinical Chemistry and Department of Internal Medicine, University of Oulu, Oulu, Finland

The activity of uracil-DNA glycosylase, a repair enzyme for the excision of uracil from DNA, was studied systematically in different types of blood and bone marrow cells in normal individuals, in megaloblastic states, in hematological malignancies and in established human leukemia cell lines. A total of 164 subjects were investigated. One hundred and nineteen peripheral

blood (PB) and 61 bone marrow (BM) samples were obtained for enzyme determination. Isolation of different hematopoietic cells was principally done by using density-dependent centrifugation techniques. Böyum's single gradient method was used for PB mononuclear and also for BM cell separation. Narrow range/stepwise density gradient centrifugation on Percoll was used for enrichment of reticulocytes and BM cells of different maturation stages. Monocytes were isolated from lymphocytes by utilizing the adhesive properties of the cells. Subpopulations of lymphocytes were separated using monoclonal antibodies and a panning technique and rosetting cells with sheep red blood cells.

The uracil-DNA glycosylase assay was based on measuring the removal of acid-soluble radioactive uracil from substrate DNA, which was synthesized from DNase I-activated calf thymus DNA by nick translation in a cocktail containing [5-^{3}H]deoxyuridine triphosphate.

The highest uracil-DNA glycosylase activities were found in the most primitive cells of benign and malignant hematopoiesis. The expression of uracil-DNA glycosylase gradually diminished towards the more mature cells. This was observed in normal bone marrow, in chronic granulocytic leukemia and in cultured malignant histiocytes, when the latter were induced to terminal maturation by phorbol ester. Blood immunocompetent cells; lymphocytes and monocytes in healthy persons, had higher uracil-DNA glycosylase expression than other mature cells, such as erythrocytes, granulocytes and platelets. The excision repair capacity of uracil-DNA glycosylase was well maintained in human peripheral blood mononuclear cells throughout life, from the neonatal period to old age.

Transient uracil incorporation into DNA may have a role in the cellular abnormalities associated with megaloblastic hematopoiesis. The present findings demonstrated that enzymatic activity required for rapid removal of uracil from DNA is also expressed in megaloblastic states.

The integrity of genetic information is well protected by uracil-DNA glycosylase in different forms of acute leukemia. Moreover, malignant cells in chronic lymphoproliferative disorders have a normal or even increased capability of repairing DNA, at least as measured by this enzyme. Enzyme expression was equal in benign and malignant hematopoietic progenitor cells: no selectivity towards malignant versus benign progenitors can be expected in possible chemotherapeutic approaches relying on uracil-DNA glycosylase.

September, 1987

Cancer in children. The influence of some environmental factors

M. STJERNFELDT

Department of Pediatrics, Linköping University, Linköping, Sweden

Although therapeutic advances over the last 30 years have reduced the mortality due to childhood cancer, still only half the children afflicted are cured. Preventive measures through the elimination or reduction of carcinogenic agents or the encouragement of protective factors must be among the main weapons in the battle against cancer.

To elucidate some aspects of the influence of environmental factors in childhood cancer, a case-control study regarding exposure to various potential noxious factors during pregnancy was performed. Three hundred and five children with cancer were compared with 340 children with diabetes mellitus. The results indicate that there is a dose–response relationship between the number of cigarettes smoked per day by the mother during pregnancy and the risk of acute lymphoblastic leukemia in the offspring. When cigarette exposure was combined with exposure to diagnostic x-rays during pregnancy the increase in risk was more than three-fold. No similar trend was seen for solid tumours. Potential confounding factors were taken into account.

The levels of gamma radiation and radon daughter concentrations were measured in the homes of 15 children with cancer and compared to the levels in the homes of healthy control children. Measurements were made in all dwellings in which the child had lived from the time of conception to diagnosis. The levels corresponded to the national average.

Serum selenium levels at various stages of acute lymphoblastic leukemia were studied in 24 children. Children who later relapsed had lower serum selenium levels than those who remained in remission. The selenium levels were also elevated in children who had their treatment discontinued. If a low selenium level has causal implications in the course of acute lymphoblastic leukemia or is merely of secondary nature needs to be further elucidated.

October, 1987