preliminary results do not show an increase in the early or late apoptosis of CD34+ cells, following cryopreservation. We hope to standardise this method for routine evaluation of CD34 + cell viability of the grafts to be used in haematopoietic transplantation. This work was supported by a grant from CFICS n°266/99 - Ministério da Saúde

360 POSTER

Relationship between immune abnormalities post-high dose chemotherapy with stem cell support in patients with solid tumors and tumor type and stage

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Background: High-dose chemotherapy (HDC) with stem cell rescue induces profound immunosupression. Recovery of cell-mediated and antibody-mediated immunity takes 1-2 years and inversion of CD4/CD8 ratio persists for at least 1 year. Infusion of peripheral blood derived hematopoietic stem cells(PBSC) results in faster recovery of blood counts than bone marrow infusion. Is immunological recovery also faster?

Aims: We have evaluated immunological recovery after HDC+ PBSC and factors influencing immune recovery.

Patients and methods: Lymphoid subpopulations in peripheral blood were quantified by flow-citometry using surface markers CD3, CD4, CD8, CD19 and CD56. IgG, IgA, IgM and IgE concentrations were also measured. These parameters were measured 1, 2, 3, 6, 9, 12, 145, 18, 21 and 24 months (mo) after PBSC infusion in 41 consecutive patients (p) (9 males ans 32 females) treated with HDC+PBSC at our institution for metastatic breast cancer (20 pts), non-metastatic high-risk breast cancer (>10 axillary nodes or stage III) (10 pts), non-Hodgkin's lymphoma (7 pts) or other solid tumors (4 pts).

Results: The duration of cellular and humoral immune recovery was markedly different according to tumor type and stage but not to the number of CD34+ cells infused. As for cell-mediated immunity, median time to CD4/CD8 >0.8 was 3 mo (range 2-6) for pts with non-metastatic breast cancer versus 9 mo (2-24+) for metastatic breast cancer ($p<.05$), 6 mo (1-9+) for non-Hodgkin's lymphoma and 6 mo (1-8+) for other tumors.

Conclusions: Cell-mediated immune recovery after HDC+PBSC is faster than that reported for bone marrow infusion and differs according to tumor type and stage.

Growth factors/cytokines

361 POSTER

High incidence of thrombosis using G-CSF in the treatment of chemotherapy-induced neutropenia

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Background: G-CSFs are widely used as potent myelopoietic stimulators. However, its activity is not restricted to the myelopoietic system and several observations suggest that G-CSF could interfere with the hemostatic balance. The prevalence of thrombosis in cancer patients has been estimated as up to 15%. In order to assess the interaction between G-CSF and hemostasis, a retrospective analysis, was performed on 409 patients, treated with G-CSF and chemotherapy from 1996 to 2000.

Patients and Methods: 287 were females and 122 were males, the mean age was 63,4 years, (range 43-76). No significant risk factors were detected; All pts had normal renal, hepatic and hematologic function and were divided in three groups according to the number of total treatments with subcutaneous G-CSF at the standard dosage of 5 mg/kg. In the first cohort (A) (n=188, 46%) pts were treated from 1 to 5 administrations of G-CSF, while the second (B) (n=135, 33%) and third cohorts (C) (n=86, 21%) respectively received from 6 to 10 and over 10 administrations. Thrombosis events occurred in 154 patients (37,65%) distributed as follows: Cohort (A): 18,18%, Cohort (B): 25,32%, Cohort (C): 54,49%.

Results: Thromboembolic complications of central venous catheter were observed in 57,8% of patients, while thrombosis of intra-arterial catheter and deep vein thrombosis were respectively 3,25% and 31,8% of cases. Seven patients (4,55%) developed subclavian vein thrombosis, pulmonary embolization in 1,95% of cases and in only one patient (0,65%) autopsy revealed acute multifocal cerebral venous thrombosis.

Conclusions: These observations indicate that G-CSF administration may induce a higher risk of thrombosis and a careful monitoring of the venous circolation should be done.

362 POSTER

IL-2 effect on NK cell phentoype of PBPC from healthy donors and patients

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Clinical studies have demonstrated that culturing PBPC in IL-2 enhances the generation of killer cells capable of lytic activity against malignant cells. The aim of this study was to evaluate the expression of NK associated markers, adhesion molecules and killer and activation-related receptors on CD56bright and CD56dim NK cells, before and after incubation of PBPC with IL2.

PBPC from 6 healthy donors (HD) and 4 patients were cultured with IL2 for 24h and studied by flow cytometry using 4-colour staining with anti-CD3 and anti-CD56, which allows the selection of NK cells defined as CD3- and CD56+, and two additional markers.

In HD, the majority of NK cells presented, before and after incubation, a CD56dim phenotype, whereas only 5% and 7%, respectively, was CD56bright. Although these two NK populations share several surface markers, statistically significant differences ($p<0,05$) were observed between them: pre-incubation -CD11c, CD16, CD57, CD94, CD158a, Granzyme B (GB) and HLA-DR; post- incubation - CD2, CD11c, CD16, CD57, CD94, CD158a, GB and HLA-DR. Following IL2 incubation there were differences within each CD56+ population: CD56brihgt cells showed an increased % of CD2+ and GB+ cells and a decreased % of CD161+ cells, whereas CD56dim cells demonstrated an increased % of CD69+ and NKb1+ cells and a decreased % of CD16+ cells.

Similar to HD cells, patient NK cells present a different phenotypic pattern for CD56bright and CD56dim cells. Comparing CD56+ populations of HD and patients we were able to detect various differences: pre-incubation - patient CD56dim cells demonstrated an increased % of CD16+ and CD94+ cells while CD56bright cells showed a decreased % of HLA-DR+ cells; post-incubation - whereas no discrepancies were encountered in CD56bright cells, the CD56dim population had an increased % of CD16+ and CD94+ cells and a decreased % of CD158a+ and GB+ cells. In what concerns pre-post incubation, a significant increase of CD69+ cells was observed in both CD56+ populations of patient NK cells.

Our preliminary results indicate that a 24 h incubation with IL-2 induces an increased % of CD69+ cells, not only in HD but also in patient NK cells. These results seem to agree with the function of CD69, one of the earliest activation markers acquired during NK cell activation. While comparing results of HD with those of patients, we were able to observe a differentiated pattern of some surface antigens specific for NK cells. Determining to what extent these cell surface receptors are functionally significant in NK cells will depend on further investigation, namely cytotoxic essays.

Head and neck cancer

363 POSTER

Limited dose external beam irradiation and interstitial iridium192 implant in definitive treatment of carcinoma of the oropharynx. Long term results

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Purpose: To evaluate long term treatment results of definitive radiation therapy in the treatment of carcinoma of the oropharynx.

Materials And Methods: 215 patients with biopsy-proven carcinoma of the oropharynx were treated during January, 1979 to October, 1995 at Long Beach Memorial Medical Center, California. There were 132 males and 80 female patients with median age of 60 (range 24 to 82 years). Forty-two patients had stage II disease and 173 patients had stage III/IV (AJCC) tumors. The external beam irradiation included the primary site as well as

entire neck to a dose of 45 to 50.4 Gy over 5 to 5 1/2 weeks time. The interstitial implant to original tumor volume with 2 to 3 cm margins were performed under general anesthesia 2-3 weeks after completion of external beam irradiation. An interstitial implant boost varied according to the stage of disease, 20 to 40 Gy at dose rate of 40 to 50 cGy per hour.

Results: Overall local tumor control was achieved in 90% of patients and overall neck control was achieved in 91% of patients. Five year disease-free survival (Kaplan-Meier) for stage II disease was 85%, for stage III disease 75% and for stage IV 56.25%. The overall disease-free survival as well as overall survival for the entire group at five years were 77% and 40%, respectively. RTOG grade III and IV late sequelae occurred in 7.8% and < 2% of patients, respectively. The majority of patients had excellent cosmetic and functional outcome.

Conclusion: The combined modality including limited dose of external beam irradiation followed by interstitial brachytherapy in the treatment of carcinoma of the oropharynx yields excellent long term disease control with acceptable treatment-related morbidity as well as preservation of cosmesis and functional integrity.

364 POSTER

Is there a prognostic influence of tumor oxygenation measured after radiotherapy in patients with SCCHN?

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Purpose: Recent experimental data in SCCHN (squamous cell carcinoma of the head and neck) in nude mice of A. Ressel et al. (IORBP, No.4; 2001) showed a good correlation between the posttherapeutical fraction of polarographically measured hypoxic values and tumor response. In our present study we retrospectively evaluated in 39 patients whether a comparable effect can be observed in the clinical situation.

Patients and methods: The oxygenation status was polarographically (Eppendorf histograph) determined 3 weeks after the onset of treatment (30 Gy) and after the end of treatment (70 Gy). Patients were treated with radiotherapy alone (5x2 Gy/week, 70 Gy, n=11) or with radiochemotherapy (5x2 Gy/week, 70 Gy, mitomycin C, 5-FU, n=28). At 70 Gy we could perform measurements in only 19 patients due to tumor shrinkage under therapy.

Results: In the univariate analysis neither the polarographic hypoxic fraction (HF<5mmHg, p = 0,6) nor the median pO2 (p = 0,8) after 30 Gy had any relevance for the overall survival of our patients. At 70 Gy also no influence of these two factors (HF<5mmHg: p = 0,4; median pO2: p = 0,4) on overall survival could be observed.

Conclusion: In contrast to the experimental findings of A. Ressel et al. we observed no influence on overall survival of the oxygenation status during and after treatment. Furthermore, Ressel et al. observed an increase of the pO2 during therapy. However, we previously described under clinical conditions a decrease of the pO2 after 30 and 70 Gy, respectively (Stadler et al. Radiother. Oncol. 1998). Therefore, we conclude that these experimental data do not reflect the clinical situation.

365 POSTER

CT-based target contouring of the primary site in a prospective head and neck cancer trial: significance for a resident training program

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Introduction: In meta-analyses of Head and Neck Cancer, concurrent chemoradiation and altered fractionation schemes have shown to improve locoregional control and survival at the cost of increased toxicity. 3-D CRT techniques are currently implemented in sparing of normal tissues. Radioprotectors, like Amifostine, may further increase the therapeutic ratio. However, of paramount importance for the outcome is the adequacy in delineation of the target. In Rotterdam we started a prospective clinical trial of concomitant chemoradiation, randomized for Amifostine prior to RT. Of all primary tumor sites, e.g. larynx (L), piriform sinus (PS), base of tongue (BOT), tonsillar fossa/soft palate (TF/SP), the target was delineated independently by a resident in training and two senior radiation-oncologists on CT.

Patients and Methods: A treatment protocol was designed and guidelines were given for the standardization of the CTV of the (elective) neck nodal regions. No strict guidelines were given for the delineation of the primary tumor site. Per primary tumor site 3 patients were analyzed. The CT-based (MRI-matched) delineation of the primary targets and neck nodal regions was performed by an experienced resident (MB); contours were checked and modified by the physician in charge (senior staffmember) before the start of the actual 3-D treatment planning process. For the purpose of this investigation, the initial set of contours (MB) was saved, checked and modified a second (PL) and a third (PN) time. The contouring by PL, PN, being senior H&N radiation-oncologists, was solely for comparison purposes, that is without consequence to the treatment per se. An analysis was performed regarding the 3 sets of contours (MB, PL, PN).

Results and Discussion: The common volumes of the primary target for MB, PL and PN were quite similar, with little variation per site: MB-PL 85%, MB-PN 87%, PL-PN 86%. However, it is uncertain whether the missed volume (MB-PL 13%, MB-PN 12%, PL-PN 14%) is of clinical relevance. This has to do with the poor resolution of CT/MRI in e.g. BOT and TF tumors, but also due to lack of standardization. The first problem might be very difficult to solve at this time and age. With regard to the second problem: for conformal therapy treatment it is mandatory to provide the clinician with rigid guidelines for the primary tumor site, based on CT and MRI.

366 POSTER

Molecular detection of tumor cells in pharyngo-esophageal brush from patients with head and neck squamous cell carcinoma

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Pharyngo-esophageal brush-capsule (Oesotest®) with cytological analysis is a simple noninvasive technique for early detection of metachronous and recurrent head and neck squamous cell carcinoma (HNSCC). Microsatellite instability (allele shift) at tetranucleotide repeat markers is a clonal marker of HNSCC. We tested whether this marker could increase the sensitivity of oesotest® for detection of rare tumor cells, compared to cytological analysis.

A series of 56 patients with untreated HNSCC had an oesotest® before initial treatment. All these patients had an oesophagoscopy during endoscopy and no additional esophageal tumor was found. Our hypothesis was that oesotest® could collect rare exfoliated cells of the primary HNSCC tumor. Microsatellite instability with marker UT5085 was observed in only 14 of 56 (25%) primary HNSCC. Cytological examination with Papanicolaou staining and molecular analysis were compared for these 15 patients.

Cytological analysis could detect tumor cells in 6 out of 14 (43%) patients. Microsatellite instability was observed in 11 out of 14 (78%) of the same sampling (p=0.03). All cytologic-positive samples were also positive with molecular analysis.

Though cytological examination remains the standard method, this study suggest that molecular analysis could greatly increase the sensitivity of oesotest®. This study also emphasizes the need of other molecular markers in HNSCC

367 POSTER

Late toxicity in three fractionation schedules for advanced laryngeal cancer

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Purpose: Accelerated radiotherapy may improve locoregional control in advanced laryngeal cancer, however, late toxicity may increase. We compared late toxicity between two accelerated and a conventional fractionation (fr.) schedules.

Methods: Primary radiotherapy was performed in 132 patients with advanced laryngeal cancer, follow-up is at least 6 months. Sixty patients (1981-1990) were treated with conventional fr. (Cfr: 50 Gy elective dose, 70 Gy tumour dose; 5 x 2 Gy weekly); 29 patients (1994-1997) were treated with combined hyperfractionation (week 1-3: 30 x 1.2 Gy) and accelerated fractionation (week 4-5: 20 x 1.7 Gy): HAfr. (elective dose 53 Gy, tumour dose 70 Gy in 5 weeks); 43 patients (from 1997) were treated with concomitant boost: Afr. (week 1-2: 10 x 2 Gy, week 3-5: 15 x 1.8 Gy elective field and 15 x 1.5 Gy boost field; 47 Gy elective dose, 69.5 Gy tumour dose in 5 weeks). Interval between fractions was at least 6 hours. Field sizes were comparable between the three groups.

Results: For patients with local control severe laryngeal oedema, requiring intervention, was seen in 6% for Cfr., 6% for HAfr., and 15% for