Boron analysis of tissue samples by quantitative neutron autoradiography and prompt gamma activation analysis in combination with histological methods

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Introduction: Due to the encouraging results of the BNCT research on non-resectable liver-metastases of colorectal carcinoma in Pavia, Italy, within recent years, a close cooperation was formed between the University of Mainz and different European institutions to assess samples of a cooperative, clinical study with up to 15 patients, which is carried out to determine the boron uptake in both healthy and tumour tissue of the liver. Four patients have been enrolled so far.

Experimental: Three methods for tissue analysis have been selected: Neutron-autoradiography using CR-39 films, prompt gamma neutron activation analysis (PGAA) and ICP-MS. CR-39 films overlaid with tissue slices containing ^{10}B are irradiated at the TRIGA Mainz and analysed after chemical etching. The method for quantitative neutron capture radiographic analysis (QNCR) was developed in cooperation with the BNCT groups in Pavia and Bremen, Germany, whereas PGAA is performed at the JRC in Petten, The Netherlands and at the TU Munich.

Table 2: B-10 concentration in tissue, µg / g (ppm)

	Patient 1	Patient 2	Patient 3	Patient 4
Hepatocytes	9*	11	7	8
Steatotic Hepatocytes	n.d.	n.d.	2 – 5 ** (3 ± 1)	4 – 7 ** (5 ± 1)
Necrotic tissue	4	3 - 9 *** (6 ± 1)	5	5
Tumour with necrotic areas	15 - 24 *** (19 ± 3)	n.d.	10 - 21 *** (16 ± 3)	n.d.
Fibrosis	n.d.	25	n.d.	19
Tumour	n.d.	28	34	20

n.d. = not determined
* mean over hepatocytes and steatotic hepatocytes together
** values varied according to dimension of steatosis
*** values varied according to dimension of necrosis

All samples are provided by the hospital of the University of Mainz. After a BPA infusion of 200 mg/kg body weight, liver samples were taken from different positions and depths of the organ, then frozen in liquid nitrogen and cut to thin slices (10-50 µm). The cell areas in tumour and tumour free tissue were characterised by the pathologist to be able to deduce a cell type dependence of the boron enrichment. The data vary greatly between the different types of cells. As anticipated, the uptake is highest for proliferating tumour tissue (20 to 34 ppm) and lowest for necrotic tissue (4 to 7 ppm), compared to an uptake of 7 – 11 ppm in tumour free liver tissue. The results are shown in table 1.

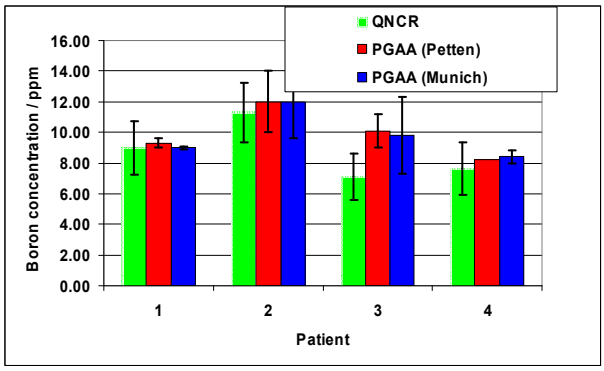

Fig 1: Results from the tissue samples of the first four patients (ppm ^{10}B), samples measured in Petten, Munich and Mainz.

Tissue samples obtained from tumour free tissue were also measured with PGAA, as to confirm the results of the QNCR analysis. Tumour samples were excluded, as during PGAA it is not possible to determine the spatial distribution of the analyte, which was crucial for our analysis. Tumour free liver tissue provides eligible samples material as it is very homogeneous in its cellular structure and composition. The analysis of the same samples with all three methods showed a good correlation, especially for the measurements with PGAA (Fig.1).
The results prove the need for an analysis including biological parameters, as the classifications of "tumour" or "tumour free" tissue do not reflect the real situation well enough for precise treatment planning. In the future, this shall be achieved including histochemical methods along with QNCR.

Acknowledgments: Christian Schütz was kindly supported by the Boehringer Ingelheim Foundation