Table 4. Dose reduction or delay

	Course					
	1	2	3	4	5	6
% of patients with any dosage reduction	6	13	13	12	16	15
% of patients with 1 week delay	—	4	7	4	11	5
% of patients with 2 weeks delay	—	6	4	6	7	10

patients became (or remained) infertile and the actuarial risk of secondary leukaemia or a second malignancy was 2.7 and 8.3% (this includes five cases of basal cell carcinoma) at 10 years, respectively. New drug combinations are currently being evaluated with the aim of reducing or preventing these long-term toxicities.

In conclusion, ChlVPP combination chemotherapy for Hodgkin's disease represents a major step forward in markedly reducing the acute toxicity seen with other drug combinations whilst maintaining the long-term remissions achieved with MOPP.

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Expression of Cathepsin D in Head and Neck Cancer

R. Zeillinger, H. Swoboda, E. Machacek, D. Nekahm, G. Sliutz, W. Knogler, P. Speiser, E. Swoboda and E. Kubista

To determine overexpression of cathepsin D in head and neck tumours we examined cytosols from 53 primary tumours, nine cytosols of lymph node metastases and 12 cytosols from adjacent normal tissue. We found a significantly lower concentration in normal tissue compared with tumour cytosol as well as with metastases, even when we compared tumours and corresponding metastases pairwise. In addition, we found a significantly higher concentration of cathepsin D in five lymph node metastases than in the corresponding tumours. We conclude that the reported role of cathepsin D is not restricted to breast cancer but could also be important in head and neck cancer.

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INTRODUCTION

CATHEPSIN D, a lysosomal acidic protease [1], possibly degrades extracellular matrix [2] when autoactivated. Thus, it may facilitate dissemination of tumours [3]. It has been reported that cathepsin D is secreted in excess by breast cancer cells compared with normal cells [4]. In clinical studies, overexpression of cathepsin D correlated with aggressive tumour behaviour, early relapse and shortened survival [5, 6]. Compared with histopathological factors, cathepsin D was an independent marker for prognosis, especially in node-negative breast cancer [5–9].

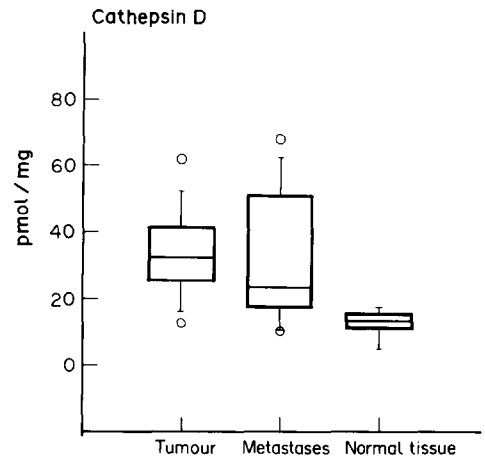

Fig. 1. Cathepsin D expression in tumours, metastases and normal tissue. Top and bottom of box = 25th and 75th percentiles, respectively, and ends of bars = 5th and 95th percentiles. Open circles = points outside 10th and 90th percentiles.

MATERIALS AND METHODS

We investigated 53 patients (48 male, 5 female) with primary squamous cell carcinoma of the head and neck with locoregional extension undergoing surgery. No patient had distant metastases. Small tumours were excluded in order not to compromise pathohistological resection margin assessment. We restricted sampling of normal mucosa to those cases where resection of normal structures was required for functional reasons (n = 12). Tumour specimens were stored in liquid nitrogen immediately after excision. For analysis, we homogenised tissue specimens using an Ultra-Turrax in phosphate buffer containing 0.1% monothioglycerol at 4°C. The homogenate was centrifuged at 50000 g for 1 h. We used the supernatant (cytosol fraction) for protein measurement. For quantification of total cathepsin D, we used a commercially available solid phase two site immunoradiometric assay (CIS Bioindustries, France). We measured radioactivity of the sandwich complex formed by two monoclonal antibodies binding to sterically remote antigenic sites of the cathepsin D molecule. The first was coated to the solid phase and the second was radiolabelled by ^{125}I.

We analysed results using the Mann–Whitney U-test for non-parametric data and the two-tailed t-test for dependent variables. We considered differences significant at $P < 0.05$.

RESULTS

In primary tumours, the mean cathepsin D concentration was 34.5 pmol/mg (S.D. 14.7). Concentrations of cathepsin D were similar in metastases (41.6 pmol/mg) whereas we found a significantly lower concentration of cathepsin D in normal tissue (13.7 pmol/mg) (Table 1, Fig. 1).

Comparing tumour specimens and normal tissue in pairs, concentrations of cathepsin D were significantly lower than in the corresponding tumour (Table 1). Additionally, we found a higher cathepsin D concentration in five lymph-node metastases than in the corresponding primary tumours (Table 1).

Table 1. Statistical analysis of cathepsin D concentrations in normal tissue, tumours and metastases

	Tumour	Metastases	Normal tissue	P
All samples	34.5 (14.7) (n = 53)	—	13.7 (4.5) (n = 12)	< 0.0001*
	34.5 (14.7) (n = 53)	41.6 (19.7) (n = 9)	—	n.s.*
	—	41.6 (19.7) (n = 9)	13.7 (4.5) (n = 12)	< 0.002*
Corresponding samples	38.5 (13.2) (n = 5)	50.44 (6.1) (n = 5)	—	< 0.04†
	36.9 (16.6) (n = 12)	—	13.7 (4.5) (n = 12)	< 0.0003†

*Mann–Whitney U test; †two-tailed t-test.

Furthermore, we compared cathepsin D concentrations with conventional markers of tumour stage. However, cathepsin D concentrations did not correlate with clinical UICC classification or pathohistological grading (WHO). Differences between 33 node-positive and 20 node-negative patients concerning cathepsin D were not significant.

DISCUSSION

The ubiquitously expressed lysosomal protease cathepsin D is initially synthesised as an inactive precursor [1]. This enzyme pro-cathepsin D is subsequently converted to its active form by proteolytic processing [10]. Transfection experiments, as well as clinical studies, indicate that overexpression of cathepsin D can increase the metastatic potential of breast cancer cells [11, 12]. The correlation of cathepsin D overexpression in breast cancer patients has been reported by several authors [5–9]. Until now, no data concerning overexpression of cathepsin D in head and neck squamous cell carcinoma were available from the literature.

Our results indicate that cathepsin D is significantly overexpressed in head and neck squamous cell carcinoma and is independent from other pathohistological markers. The high cytosolic concentration of cathepsin D in primary tumours and lymph node metastases compared to normal tissue suggests an important role of this protease in tumour invasion and metastatic potential. Thus, cathepsin D overexpression may be related with tumour dissemination of squamous cell carcinoma of the head and neck. Maybe cathepsin D is an indicator for tumour invasion and metastatic potential in different squamous cell carcinoma.

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Axillary Dissection of Level I and II Lymph Nodes is Important in Breast Cancer Classification

Christen K. Axelsson, Henning T. Mouridsen and Karin Zedeler, on behalf of The Danish Breast Cancer Cooperative Group (DBCG)

In order to define the term "a node-negative patient", the axillary nodal status at the primary operation for breast cancer was evaluated in 13851 patients registered by the Danish Breast Cancer Cooperative Group (DBCG). The determinants for node negativity in primary breast cancer were the number of lymph nodes removed and the tumour size. The number of lymph nodes removed should be at least 10 to exclude misclassification of node-positive patients as node negative. There was a strong relationship between tumour size and the percentage of node-negative patients. Another observation was that high rate of node negativity was associated with low histological grade. The age of the patients had no influence on node negativity. Where 10 or more negative lymph nodes were removed, significantly better axillary recurrence-free survival ($P < 0.0001$), over-all recurrence-free survival ($P < 0.0001$) and survival ($P < 0.005$) were found.
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INTRODUCTION

AXILLARY NODE negativity in primary breast cancer has paramount significance for staging of the disease, and thereby for the prognostic evaluation and the planning of the adjuvant treatment. But what is a "node-negative patient"? Usual definitions focus on the number of lymph nodes excised at operation exclusively, but there is no common agreement with respect to the desirable number of lymph nodes to remove [1]. Furthermore, the therapeutic aspect and the complications to axillary lymphadenectomy are still a topic for debate.

The present paper describes the experiences gathered in the register of The Danish Breast Cancer Cooperative Group (DBCG)—a nationwide programme for treatment of primary breast cancer.

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MATERIALS AND METHODS

DBCG was established in 1976. The structure of the organisation and a detailed description of the inclusion into protocols has been given elsewhere [2].

Surgical treatment

The primary surgical treatment was total mastectomy or, in a minor proportion, breast conserving treatment. Both operations were performed in connection with a partial axillary dissection.

Postoperative treatment and follow-up

Patients with no evidence of distant metastases as evaluated by physical examination, X-ray of chest, and bone scintigraphy or X-ray of the central skeleton, entered prospective trials of adjuvant therapy and follow-up (the DBCG 77 and 82 programs) [2]. High risk patients (tumour > 5 cm, and/or positive nodes, and/or skin/fascia invasion) received adjuvant systemic therapy and/or radiotherapy. Low risk patients (tumour 5 cm, and negative nodes, and no skin/fascia invasion) were observed and received no adjuvant therapy.