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Macroprolactinomas and functionless pituitary tumours

Immunostaining and effect of dopamine agonist therapy

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Abstract. In 32 patients with macroprolactinomas or functionless pituitary macroadenomas biochemical and clinical data were correlated with PRL immunocytochemistry. Serum PRL levels revealed a positive correlation with tumour PRL content. Hyperprolactinaemia of 3000 mU/l or more was found only in patients with PRL-positive tumours. In 15 patients with borderline hyperprolactinaemia (below 3000 mU/l), 7 PRL-positive and 8 PRL-negative macroadenomas were found, and in 9 normoprolactinaemic patients 4 PRL-positive and 5 PRL-negative macroadenomas. Patients with PRL-immunostainable tumours had significantly higher median basal serum PRL ($P \leq 0.05$) than patients with PRL-negative tumours. PRL stimulation after TRH, basal and GnRH-stimulated FSH and LH did not show significant differences between the two groups. A discriminant analysis using 6 biochemical variables was attempted to differentiate between PRL-negative and -positive tumours, which would be helpful in patients with borderline hyperprolactinaemia. Dopamine agonist therapy led to suppression of serum PRL with few exceptions in patients with PRL-positive and -negative tumours, whereas shrinkage was only observed in PRL-immunostainable tumours with high serum PRL levels (over 18 000 mU/l). All patients with PRL-negative tumours showed no change or even growth of the tumour despite dopamine agonist therapy. Our observations indicate that a pituitary macroadenoma associated with serum PRL of more than 3000 mU/l is most probably a prolactinoma (tumour immunostainable for PRL). Dopamine agonist therapy is effective in PRL suppression and tumour shrinkage in most of these patients. Macroadenomas without hormone hypersecretion or with borderline hyperprolactinaemia below 3000 mU/l may or may not contain PRL-immunostainable cells. Dopamine agonist therapy is effective in suppression of serum PRL also in most of these patients, but definite tumour shrinkage was not observed even in patients with PRL-immunostainable tumours of this group.

At present it appears impossible to differentiate prolactinomas and functionless pituitary adenomas with secondary hyperprolactinaemia due to pituitary stalk obstruction ('pseudoprolactinomas') on the basis of basal PRL values or dynamic tests of PRL secretion (Nabarro 1982; Grossman & Besser 1985; Randall et al. 1985). Prolactinomas can be treated successfully by dopaminergic drugs (Molitch et al. 1985), whereas in functionless adenomas no relevant tumour regression owing to dopamine agonist therapy could be observed (Barrow et al. 1984; Grossman et al. 1985; Zárate et al. 1985). Therefore, in the present analysis we attempt to differentiate the two conditions by retrospectively analysing 32 patients with macroprolactinomas or functionless pituitary macroadenomas, in whom PRL immunocyto-

chemistry of the tumour was available. The effect of dopamine agonist therapy was evaluable in 19 out of the 32 patients.

Patients and Methods

Thirty-two patients with pituitary macroadenomas (10 mm or more in diameter) were studied. The preliminary classification before operation and immunocytochemical evaluation was prolactinoma in 9 patients (5 females, 4 males) presenting with basal serum PRL values of 3000 mU/l or more and functionless adenoma with normoprolactinaemia or with moderate secondary hyperprolactinaemia below 3000 mU/l in 23 patients (10 females, 13 males). Transfrontal or transsphenoidal operation of the pituitary tumour had been performed in all 32 patients; indications for surgery were the size of the tumour and/or neurologic symptoms and/or intolerance as well as ineffectiveness of dopamine agonist therapy.

Assessment of endocrine function as evaluated in this study included: basal serum PRL (normal < 300 mU/l for men and menopausal women, < 500 mU/l for premenopausal women; correlation to S.I. unit: 1 mU = 32.9 ng PRL); stimulated serum PRL, 15 and 30 min after 200 μg of TRH iv; basal serum FSH and LH and stimulation of FSH and LH with 100 μg of GnRH iv. Complete endocrine assessment was not available for all the patients in this retrospective study. Radiological investigation of the sella had been carried out by means of CT-scanning using direct coronal views, if feasible. The majority of CT examinations were done on a Siemens DR2 scanner with a matrix of 256 × 256.

Tumour specimens had been investigated for conventional histology (standard haematoxylin-eosin staining); immunocytochemical staining was performed as follows: sections of formalin-fixed and paraffin-embedded tumour specimens were mounted on poly-L-lysine coated slides and placed in an incubator at 56°C, transferred directly into a xylene bath and rehydrated. After incubation with pronase (0.1%, 10 min) the sections were immunostained for PRL and GH by the indirect peroxidase technique (Polak & Van Noorden 1983). For this procedure the following test kits were used: Histoset immunoperoxidase staining kit for PRL (Ortho Diagnostic Systems Inc, Raritan, NJ) and Histo Scan™ immunoperoxidase staining kit for GH (Biomedica Corp, Foster City, CA). Sections incubated with phosphate-buffered saline (PBS) instead of the primary antibody served as negative controls. Staining sections were evaluated semi-quantitatively and tumours classified as containing 0%, 1–5%, 5–30%, 30–60% and 60–100% PRL-positive cells.

The statistical significance test used was the Mann-Whitney U-test. A discriminant analysis was performed with a BMD Biomedical Computer program (Dixon 1967), using the Mahalanobis generalized distance (Mahalanobis 1936).

Results

Clinical data, conventional histology, and PRL immunostaining, as well as basal and TRH-stimulated PRL are given in Table 1 for the 32 patients with prolactinomas or inactive adenomas. As can be seen, 19 tumours were immunostainable for PRL, whereas 13 tumours did not contain PRL-immunostainable cells. A semi-quantitative grading of the number of PRL-containing cells within the tumour was performed. Immunostaining for GH (not shown in Table 1) was negative in 29 tumours; sporadic GH-positive cells (< 1%) were observed in only 3 chromophobe adenomas. PRL immunostaining was negative in one of these tumours, whereas in the other two cases 1–5% and 5–30% PRL-positive cells, respectively, were found.

Four out of 13 PRL-negative and 5 out of 19 PRL-positive tumours were treated with a dopamine agonist pre-operatively.

A correlation of basal serum PRL levels with semi-quantitative tumour PRL cell content could be demonstrated. Patients with tumours containing more than 30% PRL-positive cells all had basal PRL levels of more than 6000 mU/l (range 6779–249 000), whereas in patients with PRL-negative tumours, serum PRL levels of a maximum of 2500 mU/l (range 137–2432) were observed. Patients with tumours containing only 1–30% PRL-positive cells had basal serum PRL levels ranging from 27 up to 24 800 mU/l. Hyperprolactinaemia of 3000 mU/l or more was found only in patients with PRL-positive tumours. In 15 patients with borderline hyperprolactinaemia up to 3000 mU/l, 7 PRL-positive and 8 PRL-negative macroadenomas were observed. In 9 normoprolactinaemic patients, 4 PRL-positive (1–30%) and 5 PRL-negative macroadenomas were found.

The group of patients with PRL-negative tumours was compared with the group with PRL-positive tumours. Age, basal PRL and PRL stimulation with TRH for both groups are listed in Table 2. Age at clinical presentation was not significantly different in the two groups of pa-

tients. As expected, the median basal serum PRL value was significantly higher in patients with PRL-positive tumours. The median value of increase of PRL over basal values after TRH stimulation was 91.5% in patients with PRL-negative and 35% in patients with PRL-positive tumours with an overlap between the two groups; the difference was not statistically significant ($P \leq 0.10$).

Table 1.

Clinical data, histology, immunocytochemical staining for PRL, basal and TRH-stimulated serum PRL in 32 patients with prolactinomas or functionless pituitary tumours. Age at diagnosis; histology: chr = chromophobe, eo = eosinophil, baso = basophil. PRL levels were determined pre-operatively without dopamine agonist therapy except in patients characterized with (3) in Comments; PRL/TRH: maximal PRL stimulation with 200 μg of TRH iv after 15 or 30 min (% above basal level); Comments: (1) basal PRL before second operation; (2) in these patients with recurrent tumours re-excision was not performed and therefore histology and PRL-immunostaining of the tumour of the previous operation was used in this study; (3) basal PRL with dopamine agonist premedication; (4) histology of recurrent tumour was eosinophil.

Patient	Sex	Age	Histology	PRL immunocytology (% positive cells)	Dopamine agonist preoperatively	Basal PRL (mU/l)	PRL/TRH (%)	Comments
1	M	48	chr	0	no	334	–	recurrent tumour 3 years later (4)
2	M	20	chr	0	no	900	31	
3	M	42	chr	0	no	252	141	
4	M	43	chr	0	no	190	58	
5	M	50	eo	0	yes	219	150	
6	M	40	chr	0	no	137	524	
7	M	30	chr	0	no	311	125	
8	F	76	chr	0	no	1088	37	menopause
9	F	61	chr	0	yes	1400	57	menopause
10	F	38	chr	0	no	170	–	menopause recurrent tumour (1)
11	F	37	chr	0	yes	2432	–	
12	F	37	chr	0	no	1355	181	
13	F	23	chr	0	yes	882	28	
14	M	65	eo	1– 5	no	27	70	
15	M	35	chr	1– 5	no	1000	20	
16	M	57	chr	1– 5	no	392	33	
17	M	38	chr	1– 5	no	358	81	recurrent tumour (2)
18	M	48	chr	1– 5	yes	18 440	11	
19	F	40	chr	1– 5	no	30	–	
20	F	31	eo	1– 5	no	3000	–	recurrent tumour (2)
21	F	29	chr	1– 5	yes	1650	61	
22	M	53	chr	5– 30	no	700	100	
23	M	47	chr	5– 30	no	650	30	
24	F	40	chr	5– 30	no	305	–	
25	F	35	chr	5– 30	no	445	252	
26	F	45	chr	5– 30	yes	24 800	35	
27	M	28	chr	30– 60	yes	65 000	–	recurrent tumour (1), (3)
28	M	36	chr	60–100	no	170 000	–	
29	M	30	chr	60–100	no	249 000	–	recurrent tumour (1)
30	F	20	chr	60–100	yes	95 000	0	
31	F	38	chr	60–100	no	6779	–	
32	F	24	chr + baso	60–100	no	7500	–	recurrent tumour (2), (3)

Table 2.
Age, basal PRL and PRL stimulation with TRH in patients with tumours which are negative and positive for PRL immunostaining. For detailed data see Table 1. PRL/TRH: see legend to Table 1.

PRL-immunostaining	Negative	Positive
N	13	19
Male/female	7/6	10/9
Age (years), $\bar{x} \pm$ SEM	41 ± 4	38 ± 2
Basal PRL (mU/l)[a]	334 (137–2432)	1650 (27–249 000)[b]
PRL/TRH (%)[a]	91.5 (28–524)[c]	35 (0–252)[d,e]

a: Median value and range.
b: Significantly different from the group with PRL negative tumours ($P \leq 0.05$).
c: N = 10. d: N = 11.
e: Not significantly different from the group with PRL-negative tumours ($P \leq 0.10$).

Mean basal FSH and LH values were not significantly different in the two groups, mean GnRH-stimulated LH and FSH values were higher in the group of patients with PRL-positive tumours, but the difference was not statistically significant. LH and FSH values of menopausal women were not used in the statistical evaluation because of the additional influence caused by loss of ovarian function.

Normal or moderately elevated basal serum PRL values are found in patients with PRL-negative and -positive tumours. No clear differentiation in a single case is possible by the TRH/PRL stimulation test. Therefore, in a retrospective discriminant analysis we attempted to evaluate whether a tumour was immunostainable for PRL or not. The discriminant analysis was performed between two groups (PRL-negative and PRL-positive tumours) using the following six variables: v_1 = basal serum PRL, v_2 = maximal stimulation of PRL by TRH, v_3 = basal serum FSH, v_4 = maximal GnRH-stimulated FSH, v_5 = basal serum LH, v_6 = maximal GnRH-stimulated LH. Seventeen patients (7 with PRL-negative, 10 with PRL-positive tumours, men and premenopausal women) for whom all six variables were available could be evaluated in the discriminant analysis. The probability of assignment (P, given in %) to the PRL-negative or PRL-positive group was calculated for each patient. All 7 patients with PRL-negative tumours were correctly identified in this retrospective analysis ($P > 50\%$). Seven out of the 10 patients with PRL-positive tumours were correctly identified ($P > 50\%$); 5 of them had normal or moderately elevated serum PRL levels. Three patients could not be identified as having PRL-positive immunostaining tumours ($P \leq 50\%$) in this discriminant analysis.

Response to pre- or post-operative dopamine agonist therapy was evaluable in 19 patients. The drug mainly used was bromocriptine (Parlodel®, Sandoz), given at various oral doses between 2.5 and 40 mg daily; two patients had been treated with 50 mg of bromocriptine depot im. One patient in the group with PRL-negative tumours had been treated with CU-32 (Sandoz); bromocriptine was replaced by CU-32 for a time in one patient in the group with PRL-positive tumours. As shown in Fig. 1, serum PRL was suppressible in nearly all patients with PRL-negative and -positive tumours (in 7 out of 9 patients with PRL-negative tumours, in 9 out of 10 patients with PRL-positive tumours). The effect of dopamine agonist therapy on pituitary tumours, as evaluated by CT-scan, was as follows (Fig. 1): in PRL-negative tumours (9 evaluable patients), tumour regression was not observed, there was no change of the tumour in 5, and tumour growth in 4 patients. In 9 evaluable patients with PRL-positive tumours, shrinkage of the tumour was observed in 5 patients with high serum PRL, no change in one, and growth of the tumour in 2 patients, one of whom (No. 27) developed multiple intracranial metastases in the later course (published previously) (Gasser et al. 1985). One patient (No. 17) developed central cystic regression of a recurrent

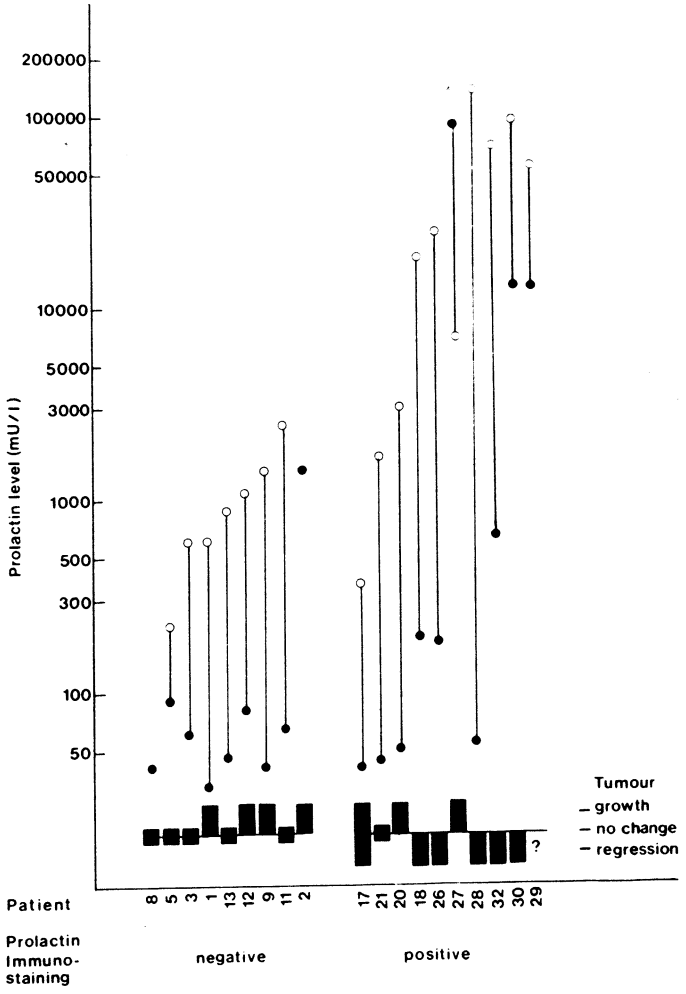

Fig. 1.
Effect of dopamine agonist therapy on serum PRL and pituitary tumours in 19 patients in correlation with PRL immunostaining of the tumour. Serum PRL before (o) and after (•) dopamine agonist therapy. ? tumour response to dopamine agonist therapy not documented.

tumour after 23 months' treatment with 5 mg of bromocriptine daily, but tumour growth was again observed after a further 11 months' treatment at the same dose.

Discussion

In a retrospective study, serum PRL and other biochemical and clinical data were correlated with tumour PRL immunostaining in 32 patients with macroprolactinomas or inactive pituitary macroadenomas.

Hyperprolactinaemia of 3000 mU/l or more was associated only with PRL-immunostainable tumours (prolactinomas). Macroadenomas associated with borderline hyperprolactinaemia (300 or 500–3000 mU/l, respectively) were either PRL-positive (moderate secreting prolactinomas) or PRL-negative (functionless adenomas with secondary hyperprolactinaemia due to pituitary stalk obstruction). However, the latter mechanism may also contribute to hyperprolactinaemia in PRL-positive macroadenomas. Even pituitary adenomas in normoprolactinaemic patients of our series could be identified as PRL-positive by immunostaining. An unequivocal distinction be-

tween prolactinomas and functionless adenomas appears to be impossible without the use of PRL immunocytochemistry. However, no patient with more than 30% of PRL-positive cells showed a serum PRL below 3000 mU/l, and this appears to be a safe criterium for the diagnosis of prolactinoma. Ross et al. (1985) and Randall et al. (1985) found that serum PRL levels greater than 6000 mU/l indicate the presence of a PRL-immunostainable pituitary tumour, whereas hyperprolactinaemia below 6000 mU/l may be associated with both prolactinomas and functionless adenomas, or other tumours of the pituitary region.

Quantitative assessment of PRL-immunostaining cells in a pituitary tumour is impaired by the occasional observation of inhomogeneous PRL immunostaining within a single tumour (Ross et al. 1985; Schatz et al. 1985). Nevertheless, a semiquantitative grading of PRL-immunostainable cells in pituitary tumours with the previously mentioned reservation seems justified to us in this clinical study, and in our series there were no great classification difficulties in this respect (Table 1). Furthermore, pre-operative treatment with a dopamine agonist may reduce tumour immunostaining for PRL (Tindall et al. 1982). This may explain the small number of PRL-immunostainable cells in the tumour of patient No. 18 in our series, who had a high serum PRL level before therapy.

In patients with moderate hyperprolactinaemia, no clear discrimination between PRL-positive and -negative tumours is possible with the usual dynamic tests of PRL secretion (Grossman & Besser 1985; Randall et al. 1985); also in our study, PRL stimulation after TRH showed a wide range of overlap between patients with PRL-positive or -negative tumours. Mean basal and GnRH-stimulated LH and FSH values were not significantly different in the two groups.

The discriminant analysis between patients with PRL-negative and -positive tumours using the 6 variables presented in this paper is an attempt to differentiate between the two conditions, in particular in patients with moderate hyperprolactinaemia. All 7 patients with PRL-negative tumours (3 with moderate hyperprolactinaemia) were correctly identified in the retrospective analysis. Seven out of 10 patients (5 of them with normal or moderately elevated serum PRL levels) were correctly identified as having PRL-positive tumours. However, the small number of evaluable patients for this analysis allows only a preliminary interpretation and the discriminant function provided here appears to be of little clinical value. We believe, however, that it would be possible to develop further improved discriminant functions on the basis of the preliminary results presented here.

The effect of dopamine agonist therapy on pituitary tumours in patients with or without hyperprolactinaemia (Fig. 1) can be interpreted in two ways: 1) normal or high serum PRL can be suppressed by adequate dopamine agonist therapy in patients with PRL-positive and -negative tumours with a few exceptions. Therefore, the clinical signs, such as galactorrhoea, amenorrhoea, or impotence can be treated successfully regardless of the aetiology of hyperprolactinaemia. 2) On the other hand, tumour shrinkage following dopamine agonist therapy was only observed in patients with immunocytochemically PRL-positive tumours and high serum PRL levels (above 18 000 mU/l in our patients). Transient cystic tumour regression after dopamine agonist therapy was only found in one patient with slight hyperprolactinaemia and a PRL-immunostainable tumour. In all patients with PRL-negative tumours, no change or even growth of the tumour despite dopamine agonist therapy was observed. Our results confirm previous studies of Barrow et al. (1984), Grossman et al. (1985), and Zárate et al. (1985).

In conclusion, our observations indicate that a pituitary macroadenoma associated with a serum PRL of more than 3000 mU/l is most probably a prolactinoma (tumour immunostainable for PRL). Dopamine agonist application is effective in PRL suppression and tumour shrinkage in many of these patients and can be recommended as primary therapy with periodical CT-scan controls except in neurological emergency situations. This is in accordance with the study of Molitch et al. (1985).

Macroadenomas without hormone hypersecretion or with borderline hyperprolactinaemia below 3000 mU/l may or may not contain PRL-immunostainable cells. Dopamine agonist therapy is effective in the suppression of hyperprolactinaemia in most of these patients and relief of some clinical signs can be achieved. However, definite tumour shrinkage after dopamine agonist treatment in these patients, even with PRL-immunostainable tumours, was not observed in our series.

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