Relationships Between Pathological Diagnosis and Clinical Parameters in Acromegaly

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From our series of 185 somatotropic adenomas with acromegaly, we found that sparsely granulated adenomas were more frequent (56%) than densely granulated ones. Immunocytochemistry detected growth hormone (GH) plurihormonal adenomas in 68% of patients. GH-α-subunit (αSU) and GH-αSU-prolactin (PRL) were more frequent (38%) than GH monohormonal adenomas (32%). The colocalization of GH and αSU in the same cell was obvious in many tumors. In contrast, colocalization of GH and PRL was demonstrated in only 25% of GH-PRL adenomas. The relationships between age, sex, tumor size, GH and PRL plasma levels, granularity, and percentage of GH-, αSU-, and PRL-immunoreactive cells were established in 105 acromegalic patients by three statistical methods, mainly by a principal component analysis. Correlations were found between the percentage of αSU- and GH-immunoreactive cells, and between densely granulated character and the percentage of GH-immunoreactive cells. Tumor size was not correlated with αSU, but was positively correlated with PRL plasma levels. Patients' age and percentage of GH-immunoreactive cells were inversely related to tumor size. Plurisecretion and sparsely granulated aspect are not related to age and tumor size.

WE REVIEWED 709 from our series of 1,400 pituitary adenomas, all studied by immunocytochemistry, which is the preferred method, giving a precise diagnosis.[1] In 185 adenomas from patients with acromegaly, we evaluated the frequency of monohormonal and plurihormonal growth hormone (GH) adenomas and the percentage of sparsely and densely granulated adenomas. In 105 patients with acromegaly, we studied nine parameters (age, sex, tumor size, GH and prolactin [PRL] plasma levels, granular aspect, and percentage of GH, α-subunit (αSU), and PRL-immunoreactive cells) to answer the question: is histological diagnosis related to tumor size, age, and clinical and biological data?

SUBJECTS AND METHODS

Subjects

All of the adenomas were obtained at surgery by the transsphenoidal route between 1974 and 1991. The patients presented with clinical signs of acromegaly. All had elevated basal plasma GH levels (5 to 388 μg/L). Responses to an oral glucose tolerance test (OGTT) were abnormal. PRL plasma levels had been determined in 87 patients by conventional radioimmunoassay. The values varied from 11 to 700 μg/L (normal values, <20 μg/L). Plasma αSU levels, obtained in 31 patients, varied from 0.3 to 21.7 μg/L (normal value, <1 μg/L). No patient had received medical treatment before surgery. Tumor size and extension were expressed by grade according to Hardy's classification.[2] For patients operated on between 1974 and 1989, grade was evaluated from the size of sella turcica and the pneumoencephalography. Thereafter, a more precise evaluation was performed by computed tomographic scan and/or magnetic resonance imaging. There were 76 small adenomas (grade I, n = 35; grade II, n = 41) and 24 macroadenomas with extrasellar extension (grade III, n = 19; grade IV, n = 5).

Histological Studies

All of the adenomas were studied by light and electron microscopy. For light microscopy, pieces of tumor tissue were fixed in Bouin-Hollande-sublimate for 4 days and embedded in paraffin. Five-micron thick sections were stained with Herlant's tetrachrome and periodic acid–Schiff (PAS)-Orange G methods.

For electron microscopy, tissues were fixed in 2% glutaraldehyde in 0.1 mol/L cacodylate buffer, postfixed in 2% osmium tetroxide, and embedded in araldite. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined with a JEOL type 1200 EX electron microscope.

For all of the adenomas, an immunocytochemical reaction was performed on contiguous sections by indirect immunoperoxidase and streptavidine-biotin complex methods as previously described.[1] A double-labeling technique was performed in 10 adenomas. Two systems of detection were used (fluorescence and peroxidase). The following monoclonal (m) and polyclonal (p) antibodies were used (at dilutions of 1/200 to 1/500 for the monoclonal antibodies and 1/1,000 to 1/5,000 for the polyclonal antibodies): anti-hPRLp, anti-hLHp (from B. Claustrat, Lyon, France), anti-hαFSHp, anti-hβTSHp, anti-hβFSHp, anti-hβLHp (from the National Institute of Diabetes and Digestive and Kidney Diseases), anti-hGHp, anti-$^{17\text{-}39}$ACTH (from M. Dubois, Nouzilly, France), anti-hTSHm (from ICN Pharmaceuticals, Costa Mesa, CA), anti-hPRLm, and two anti-hαSUm (clone 326-2-1 no. 2 and 6EA) (from Immunotech, Lumigny, France).

For each adenoma, the percentage of immunoreactive cells was obtained from the observations of two investigators on approximately 500 cells. When the distribution was irregular, the maximum and minimum percentages were noted and the mean percentage calculated. When there were discrepancies in the observations, 1,000 cells were counted instead of 500.

To determine whether GH immunoreactivity, αSU immunoreactivity, and PRL immunoreactivity were in the same cell or in different cells, homologous fields of adjacent sections immunostained by anti-hGH, anti-αSU, and anti-hPRL sera, respectively, were compared. With the double-labeling technique, the cells in which two hormones were colocalized appeared brown and green or red and green.

The possibility of cells being included from the normal pituitary was ruled out in particular by negativity of the reaction with anti-$^{17\text{-}39}$ ACTH and anti-FSH antiserum.

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Statistical Analysis

Data analyses were performed using PCSM software (Deltasoft, Grenoble, France). Nine factors were studied: age, sex, tumoral size, GH and PRL plasma levels, granularity, and percentage of GH-, αSU-, and PRL-immunoreactive cells. Possible correlations between all of these factors were analyzed by a principal component analysis.[3] This method used an orthogonal axis system in which each factor is represented by a vector. The more the directions of two projections approach parallelism, the closer the correlations. The greater the distance of a factor from the origin, the better the representation of this factor in the plane. As shown in Fig 1, the projections of the nine factors are represented on two planes corresponding to the principal axes (1,2) and (1,3). When the correlation coefficient between two factors was calculated, we always checked that a nonparametric correlation coefficient (Spearman rank) gave the same significance. Mean values were compared by the Mann-Whitney *U* or Kruskal-Wallis H test.

RESULTS

Morphological Characteristics of GH Adenomas

Using light and electron microscopy, the somatotropic adenomas were differentiated into densely or sparsely granulated adenomas. We considered densely granulated adenomas to be those with more than 50% to 60% of orangeophilic cells with Herlant's tetrachrome and with numerous secretory granules in electron microscopy. In our series, the densely granulated adenomas represented 44% of tumors.

Immunocytochemistry revealed that GH immunoreactivity was found in all the tumors. The percentage of GH-immunoreactive cells varied from 5% to 100% of total cells, but the great majority of tumors had more than 20% of GH-immunoreactive cells.

GH plurihormonal adenomas were extremely frequent, with GH-αSU ± PRL adenomas heading the list (Table 1). They represented 38% of somatotropic adenomas. They were more frequent than the GH monohormonal adenomas (32%). αSU was secreted by 48% of GH-PRL adenomas. It must be underlined that an adenoma was considered as immunoreactive for αSU and PRL when the percentage of immunoreactive cells was notable (>1%). The number of αSU-immunoreactive cells varied from 1% to 80% of total cells, but was always lower than the number of GH-immunoreactive cells. In a given adenoma, αSU was in the same cell and/or in a separate cell. With the double-labeling technique, colocalization was proved in all of the adenomas tested. In contrast, in GH-PRL adenomas, colocalization of GH and PRL was demonstrated in only 25% of GH-PRL adenomas. GH plurihormonal adenomas did not differ morphologically from GH monohormonal adenomas.

Anatomicoclinical Relationships

In GH-αSU adenomas, αSU plasma levels were increased in only 60% of patients, but it must be underlined that all of the patients with increased αSU plasma levels had αSU immunoreactivity in the tumor. In contrast, hyperprolactinemia was present in only 68% of GH-PRL adenomas.

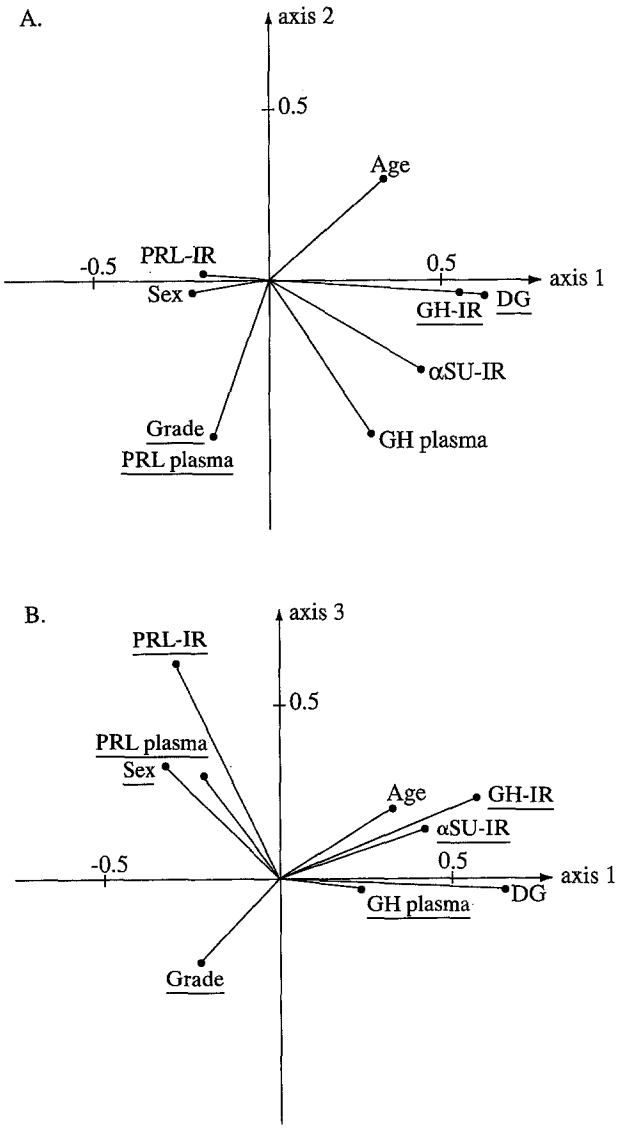

Fig 1. Graphic representation of the principal component analysis. Each histological and clinical measurement is represented by a vector, which is projected on 3 axes (24.00%, 16.33%, and 14.55% of variance). The nearer the directions of the projections, the closer the correlations. The longer the distance from the origin, the more important the role of the factor in explaining the variability of this system. The strongest relationships are underlined. (A) (axis 1, 2): A close relationship was shown between the densely granulated type (DG) and the percentage of GH-immunoreactive cells. There is also a good correlation between the percentage of GH- and αSU-immunoreactive cells. There is an inverse relationship between age and tumor grade, but the plurisecretion of GH-αSU and GH-PRL is not related to tumor size. (B) (axis 1, 3): GH plasma levels were weakly related to the tumor size, which was inversely related to the percentage of GH-immunoreactive cells.

A possible linkage between nine factors (age, tumor size, granularity, sex, percentage of GH-, PRL-, and αSU-immunoreactive cells, and GH and PRL plasma levels) was studied in 105 patients. Figure 1 shows the results of the principal component analysis. Three axes, which explained, respectively, 24.00%, 16.33%, and 14.55% of the variance, were chosen. In the first plane (axis 1, axis 2), αSU was not

Table 1. Immunocytochemical Varieties of GH Adenomas

Types	No. of Cases	(%)	
GH	60		32
GH-αSU	42	38	
GH-PRL-αSU	30		
GH-PRL	31		36
GH-TSH-PRL	4		
GH-TSH-PRL-FSH-LH	1	11	
GH-TSH	15		
GH-LH	1		
Total	185		

correlated with any factors. However, a close correlation was shown between granularity and the percentage of GH-immunoreactive cells, which were negatively correlated with the percentage of PRL-immunoreactive cells and sex. However, these last two factors were poorly represented. In the second plane (axis 1, axis 3), αSU was correlated with age and percentage of GH-immunoreactive cells. A correlation was also shown between percentage of PRL-immunoreactive cells, PRL plasma levels, and sex. Tumor size was positively correlated with plasma PRL levels and negatively with percentage of GH-immunoreactive cells. In the two planes, tumor size was negatively related to patient's age.

These observations were confirmed by examination of the correlation matrix (not shown). By multiple regression analysis, it was shown that with regression of αSU = f(other factors), no factor contributed statistically. With regression of grade = f(other factors), only PRL plasma levels statistically contributed to the regression ($P < .02$).

DISCUSSION

In our series of 105 patients with acromegaly, it was shown, by three different statistical methods, that there was a close relationship between densely granulated adenoma type and the percentage of GH-immunoreactive cells. Knowing that GH is located in the secretory granules, this is not a surprising finding. Nevertheless, there is no relationship between granularity, tumor size, and plasma GH levels. This result contradicts a correlation between the sparsely granulated character and tumor size or plasma GH levels, which has been found by several investigators.[4-7] This controversy may be explained by the short series.[4,5] No proof has been given by Kovacs and Horvath.[7] As Riedel et al[6] underlined, there is a continuum from densely to sparsely granulated adenoma. In GH adenomas, the percentage of granular cells varies from one area to another and in one adenoma, from one cell to another. In Riedel's series, which is comparable to ours (in number of cases and percentage of sparsely granulated and densely granulated adenomas), the correlation is slight. If Riedel's data are reanalyzed and the limit between densely and sparsely granulated is put at 60% of granular cells, as in our series, there is no relationship with grade. αSU immunoreactivity was slightly related to the percentage of GH-immunoreactive cells, to the densely granulated character of the adenomas, and to the GH plasma levels. No correlation was found with tumor grade. This eliminates the possibility of a correlation of αSU immunoreactivity with tumor growth.

Moreover, we found that GH plasma levels were only slightly correlated with tumor grade. So, in contrast with PRL adenomas, tumor size is not the only factor that influences levels of GH secretion. In 1980, we demonstrated the great variability of the secretory activity of GH cells from one adenoma to another.[8] Thus, we confirm the great influence of this factor in the increase of plasma GH levels.

As with Klijn et al,[9] we found a negative correlation between the size of the tumor and the age of the patient. We also found a negative correlation between tumor grade and percentage of GH-immunoreactive cells. So, if the two characteristics are taken together (granularity and percentage of GH-immunoreactive cells), there is a clear inverse correlation with tumor size and age. The low percentage of GH-immunoreactive cells alone or in association with the sparsely granulated character of the tumor is a factor in the prognosis of GH adenomas, but the sparsely granulated aspect alone is not a criterion of evolutivity. We previously underlined[10] that GH adenomas without acromegaly and low GH plasma levels (<5 μg/L) are more frequent in young patients and are larger tumors than in acromegalic patients. Thus, in general, a less-differentiated GH adenoma grows more quickly than a well-differentiated one. It is more frequent in young patients with or without acromegaly.

In GH-αSU adenomas, αSU immunoreactivity was related to the percentage of GH-immunoreactive cells and to the densely granulated character. This underlines the cosecretion of GH and αSU by the same cell.[11] Plasma αSU levels are a good marker of the disease and are an even better sign of the type of adenoma than hyperprolactinemia. Indeed, hyperprolactinemia is present in only 68% of GH-PRL adenomas. The clinician may be sure of the somatoprolactinic nature of the tumor only if plasma PRL levels are greater than 200 μg/L. The good correlation between the tumor grade and plasma PRL levels means that in many tumors the hyperprolactinemia is not related to the secretion of PRL by the tumor. PRL is secreted by the juxtatumoral pituitary. Compression of the hypothalamus by a large tumor inhibits the hypothalamic secretion of dopamine. The inverse relationship between the percentage of PRL- and GH-immunoreactive cells confirms that in many cases the two hormones are secreted by different cells. There is no correlation between tumor size and plurisecretion. This result contradicts Scheithauer et al,[12] who wrote that "plurihormonal tumors are more often macroadenomas (80%) than microadenomas (20%)." So, for us, plurisecretion does not mean a poor prognosis. It is related to the secretory pluripotentiality of the somatotropic cells. Indeed, in normal pituitaries from autopsies and surgically removed juxtatumoral pituitaries, GH and αSU are colocalized in approximately 5% of normal somatotropic cells. However, GH cells do not secrete βSU of the glycoprotein. This observation may be related to Pit_1 tissue-specific transcription factor, which regulates the expression of GH and PRL and possibly TSH and αSU genes in the normal and tumoral pituitary.

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