Angiogenesis in pituitary adenomas – relationship to endocrine function, treatment and outcome

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Abstract

Angiogenesis has been shown to be related to tumour behaviour, prognosis and response to treatment in many different tumour types. The aim of this study was to examine the relationship between angiogenesis and tumour behaviour and response to treatment in pituitary adenomas. The microvessel density (MVD) of pituitary tumours was assessed by counting blood vessels labelled with 3 different endothelial markers using antibodies to CD31, factor eight-related antigen and biotinylated *Ulex europaeus* (agglutinin I UEAI). One hundred and forty-two surgically removed pituitary adenomas (46 GH secreting, 6 microprolactinomas, 19 macroprolactinomas, 18 ACTH secreting and 53 functionless tumours) were carefully characterized and assessed. There was a significant negative correlation between age and MVD of GH secreting tumours ($R^2=33.8$, $P=0.005$). Age was not

related to MVD in other tumour types. Pre-treatment hormone production by the adenomas was related to MVD in prolactinomas ($P<0.05$), but not in GH secreting tumours. Invasive prolactinomas were significantly more vascular than non-invasive tumours ($P<0.05$). Drug treatment with metyrapone or bromocriptine did not appear to influence tumour angiogenesis. Surgical cure was more likely in macroprolactinomas and in ACTH secreting tumours with lower MVD. These results show that factors related to angiogenesis are very important in determining a number of clinical features of pituitary tumours, in particular the invasiveness of macroprolactinomas, the effect of age in tumours secreting GH and the outcome of surgical treatment in macroprolactinomas and ACTH secreting tumours.

Journal of Endocrinology (2000) **165**, 475–481

Introduction

Tumour growth beyond a few millimetres in diameter is angiogenesis dependent (Folkman 1990). In many human tumours, including breast, bladder and stomach, angiogenesis has been shown to be correlated with tumour behaviour (Weidner *et al.* 1991), outcome (Weidner *et al.* 1992, Bochner *et al.* 1995, Maeda *et al.* 1995) and response to therapy – the more vascular a tumour is, the lower the likelihood of any response to adjuvant therapy (Vacca *et al.* 1994, Gasparini *et al.* 1995, Hollingsworth *et al.* 1995).

The vascular density of different tumours has been assessed by counting vessels labelled with antibodies to different vascular endothelial markers including factor eight-related antigen (F8), CD31 (platelet endothelial cell adhesion molecule) and the lectin *Ulex europaeus* agglutinin 1 (UEAI). These markers have been shown to have different sensitivities for the detection of endothelium

(Holthofer *et al.* 1982, Horak *et al.* 1995). F8 stains large vessels but does not stain smaller microvessels (Mukai *et al.* 1980, Vermeulen *et al.* 1995), UEAI stains all microvessels and stains paraffin-embedded tissue well, but its drawback is that it stains the Golgi in some neoplastic cells (Holthofer *et al.* 1982, Witt & Klessen 1987). CD31 is a sensitive marker for all microvessels but can be difficult to use in some paraffin-embedded tissue, possibly related to antigen loss due to fixatives containing acetic acid (Vermeulen *et al.* 1996).

We have recently shown that pituitary tumours are less vascular than the normal pituitary gland. We have shown that the vascular density of microprolactinomas is significantly less than macroprolactinomas, whereas there is no difference in the vascular density of microadenomas secreting growth hormone (GH) when compared with GH secreting macroadenomas (Turner *et al.*, 2000a). In this paper, we analysed the relationships between tumours

arising from different pituitary hormone producing cells and angiogenesis. It is known that different types of pituitary tumours behave in different ways, for example silent corticotroph tumours are often more aggressive than other functionless tumours (Kovacs & Horvath 1986). Furthermore, because it is known that older patients with acromegaly have smaller tumours and have milder disease with lower GH levels than patients of a younger age at presentation, its relationship to microvascular density (MVD) was analysed (Klijn *et al.* 1980, Smals *et al.* 1988). Finally, in view of the relationship in other tumours of angiogenesis with tumour behaviour and outcome, these factors were assessed in terms of tumour characteristics at presentation, drug treatment and surgical results.

Materials and Methods

Specimen collection

One hundred and forty-two surgically removed pituitary adenomas were investigated. There were 46 GH secreting tumours (28 macroadenomas and 18 microadenomas), 6 microprolactinomas, 19 macroprolactinomas, 18 adrenocorticotrophin (ACTH) secreting tumours (Cushing's disease) and 53 non-functioning pituitary adenomas (29 gonadotroph, 21 negative and 3 silent ACTH). All the tissue had been fixed in 4% buffered formalin, dehydrated and embedded in paraffin. Histological examination and immunohistochemistry for anterior pituitary hormones had been performed previously, and together with the clinical, endocrine and radiological data were used to characterise fully each tumour type. Invasiveness was defined according to the modified Hardy criteria (Bates *et al.* 1997). Tumours with evidence of bony destruction, spread into the sphenoid and/or cavernous sinus, or tumours with central nervous system/extracranial spread on computed tomography/magnetic resonance imaging were defined as invasive. 'Cure' was defined as an undetectable post-operative cortisol value in the patients with Cushing's disease, as a prolactin value in the normal range for patients with prolactinomas, and as a mean GH value of less than 5 mU/l or suppression to less than 2 mU/l on an oral glucose tolerance test for patients with acromegaly.

Immunohistochemistry for CD31, UEAI and F8

The streptavidin-biotin peroxidase complex technique (ABC) was used for CD31 and F8, and the alkaline phosphatase/anti-alkaline phosphatase (APAAP) method was used for UEAI.

Sections (4 µm) were mounted on aptes (3-aminopropyl triethoxy silane, Sigma, Poole, Dorset, UK)-coated slides, dewaxed and rehydrated. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for CD31 and F8 antibodies. CD31 and F8 labelling required

trypsinisation (0.1% trypsin in calcium buffer) at 37 °C for 15 min. CD31 labelling required further microwave pre-treatment in sodium citrate buffer pH 6. Labelling with UEAI did not require pre-treatment.

Non-specific primary antibody binding was blocked using fetal calf serum at a dilution of 1:20 for CD31 and UEAI. The primary antibodies were all applied for 60 min at room temperature, and washed three times in buffered saline. For CD31, the DAKO antibody was applied at a dilution of 1:20. An enhanced peroxidase one step (EPOS, DAKO, High Wycombe, Bucks, UK) F8 was applied as supplied. The biotinylated UEAI (Vector Laboratories, Burlingame, CA, USA) was used at a dilution of 1:200. An anti-mouse biotinylated secondary antibody (Insight Biotechnology, Wembley, Middlesex, UK) was applied at 1:200 dilution for 30 min at room temperature to the CD31 cases. This was followed by washes, and then application of the horseradish peroxidase streptavidin complex (DAKO) at 1:400 dilution for 30 min. The APAAP complex (Vector Laboratories) was applied to the UEAI cases for 30 min followed by washes. Colour development was with metal enhanced diaminobenzidine (DAB) (Pierce and Warriner, Rockford, IL, USA) applied for 15 min to the F8 and CD31 cases, and with fast red substrate (Vector Laboratories) applied for 20 min to the UEAI cases. The slides were lightly counterstained with haematoxylin.

Assessment of vascular density

Vascular density was assessed by one examiner without knowledge of the tumour type or size. The Chalkley point technique was used as previously described (Fox *et al.* 1995, Turner *et al.* 2000a). An overall subjective semi-quantitative grading system (G) was also used (1 and 2 – low and low moderate, and 3 and 4 – high and very high vascular density). The counts (M) and grades (G) were made by a single observer (H E T), and 20% were checked by a second blinded observer (K G C). We have previously shown good intra-observer reliability for both Chalkley counts and overall grades (Turner *et al.* 2000a).

Statistical analysis

The Statgraphics software package was used. ANOVA was used for categorical data analysis and regression analysis for continuous variables.

Results

Patient characteristics – age, sex

There was no relation between patient age and vascular density of functionless tumours, macroprolactinomas and tumours causing Cushing's disease, but there was a

Figure 1 Relationship between age and vascular density in acromegaly. Axis x: vascular density as measured by CD31 expression – mean Chalkley counts; axis y: age of the patients in years. Continuous line indicates best fitting regression curve (reciprocal – Y model – $y=1/(a+bx)$); dotted lines indicate 95% confidence levels.

significant negative correlation between age and vascular density in GH secreting tumours ($R^2=22.5\%$, $P=0.017$ (UEAIM), $R^2=33.8\%$, $P=0.005$ (CD31 M)) (Fig. 1). When the relationship between age and vascular density of patients with GH secreting macroadenomas and microadenomas was analysed separately, $R^2=21.2\%$ and 73% respectively.

The sex of the patient did not affect vascular density of the different tumours (Table 1). An exception was prolactinomas where the patients with microprolactinomas were all women, and microprolactinomas were significantly less vascular than macroprolactinomas (14 males) ($P<0.05$).

Hormone level and tumour type

The pre-operative prolactin level from both microprolactinomas and macroprolactinomas was positively correlated with vascular density using both CD31 and UEAI ($R^2=61.3\%$, $P=0.003$ (CD31), $R^2=60.6\%$, $P=0.0001$ (UEAI)) (Fig. 2). This relationship persisted when

Figure 2 Relationship between preoperative serum prolactin levels and vascular density in prolactinomas. Axis x: vascular density as measured by UEAI staining – mean Chalkley counts; axis y: serum prolactin in mU/l. Continuous line indicates best fitting regression curve (exponential model – $y=e^{(a+bx)}$); dotted lines indicate 95% confidence levels.

pre-operative prolactin and vascular density in macroprolactinomas alone were considered ($R^2=35.6\%$, $P=0.02$). Cortisol and ACTH production (assessed using urinary free cortisol (UFC) and ACTH levels) were not related to vascular density of tumours causing Cushing's disease (Table 2). Pre-operative GH (mean across an oral glucose tolerance test or random) and age-related insulin-like growth factor-I were not related to vascular density of GH secreting tumours.

Tumour subtype

Tumours that stained for GH alone ($n=14$) were more vascular using ulex than those staining for GH and PRL ($n=11$) ($P=0.036$) (Table 3, Fig. 3). Although there was a trend for functionless tumours that were negative on immunostaining to be more vascular than tumours that stained for gonadotrophins, this was not statistically significant (Table 3). Silent corticotroph adenomas were the least

Table 1 Relationship between sex and vascular density. Results are means (S.E.M.). Numbers of cases in square brackets

	CD31M	CD31G	F8M	F8G	UEAIM	UEAIG
NFA-M	5.4 (0.4) [26]	2.6 (0.1) [26]	5.1 (0.5) [33]	2.3 (0.2) [33]	7.5 (0.8) [32]	3.0 (0.2) [32]
NFA-F	5.4 (0.9) [8]	2.8 (0.5) [8]	5.3 (1.0) [10]	2.4 (0.5) [10]	6.9 (1.2) [10]	2.8 (0.4) [10]
CUSH-M			4.7 (0.8) [4]	1.9 (0.6) [4]	4.8 (1.9) [4]	2.2 (0.5) [4]
CUSH-F			4.7 (0.9) [10]	1.8 (0.4) [14]	5.1 (0.9) [10]	2.3 (0.2) [11]
GH-M	5.5 (0.4) [11]	2.5 (0.5) [12]	4.6 (0.3) [23]	1.9 (0.7) [25]	7.0 (0.8) [8]	3.0 (0.5) [20]
GH-F	5.6 (0.4) [14]	2.4 (0.6) [16]	4.9 (0.3) [23]	1.9 (0.8) [26]	7.4 (0.5) [17]	2.5 (0.5) [12]

Insufficient cases of ACTH secreting tumours in males stained for CD31 for any useful statistical analysis.

M, males; F, females; NFA non-functioning tumours; Cush, ACTH secreting tumours causing Cushing's disease; GH, GH secreting tumours causing acromegaly; CD31M, mean Chalkley count using endothelial marker CD31; CD31G, semiquantitative grade using endothelial marker CD31; F8M, mean Chalkley count using endothelial marker factor eight-related antigen; F8G, semiquantitative grade using endothelial marker factor eight-related antigen; UEAIM, mean Chalkley count using endothelial marker Ulex; UEAIG, semiquantitative grade using endothelial marker Ulex.

Table 2 Vascular density and hormonal activity of tumours. Results are means (S.E.M.). Numbers of cases are in square brackets

	F8M	F8G	UEAIM	UEAIG
UFC >1000 nmol/24 h	5.1 (0.5) [5]	1.8 (0.5) [6]	5.1 (1.8) [6]	2.3 (0.5) [6]
UFC <1000 nmol/24 h	4.0 (1.4) [6]	1.7 (0.6) [8]	5.0 (1.0) [5]	2.1 (0.2) [6]
ACTH >50 ng/ml	5.4 (0.8) [4]	2.2 (0.7) [5]	5.4 (1.2) [5]	2.3 (0.4) [5]
ACTH <50 ng/ml	4.0 (1.3) [7]	1.5 (0.4) [10]	4.5 (0.8) [5]	2.0 (0.0) [6]

UFC, urinary free cortisol; F8M, mean Chalkley count using endothelial marker factor eight-related antigen; F8G, semiquantitative grade using endothelial marker factor eight-related antigen; UEAIM, mean Chalkley count using endothelial marker Ulex; UEAIG, semiquantitative grade using endothelial marker Ulex.

Table 3 Vascular density and tumour subtype. Results are means (S.E.M.). Numbers of cases are in square brackets

	CD31M	CD31G	F8M	F8G	UEAIM	UEAIG
NFA-NEG	5.4 (1.2) [19]	2.7 (0.6) [19]	5.5 (1.7) [21]	2.4 (0.8) [21]	7.9 (2.4) [19]	3.2 (0.8) [19]
ACTH	4.9 (1.2) [3]	1.8 (0.76) [3]*	5.0 (0.0) [1]	2.0 (0.0) [1]	7.2 (1.4) [3]	2.8 (0.3) [3]
Gonadotrophin	5.5 (1.0) [23]	2.7 (0.5) [23]	5.0 (1.3) [29]	2.2 (0.8) [29]	7.1 (2.2) [29]	2.8 (0.6) [29]
GH	6.0 (0.5) [11]	2.4 (0.3) [13]	4.7 (0.3) [25]	1.9 (0.3) [28]	7.9 (0.6) [14]**	3.0 (0.3) [18]
GH/PRL	5.2 (0.3) [14]	2.4 (0.2) [14]	4.9 (0.4) [18]	1.9 (0.2) [20]	6.4 (0.6) [11]	2.5 (0.2) [14]

NEG, negative; NFA, non-functioning tumours; PRL, prolactin; CD31M, mean Chalkley count using endothelial marker CD31; CD31G, semiquantitative grade using endothelial marker CD31; F8M, mean Chalkley count using endothelial marker factor eight-related antigen; F8G, semiquantitative grade using endothelial marker factor eight-related antigen; UEAIM, mean Chalkley count using endothelial marker Ulex; UEAIG, semiquantitative grade using endothelial marker Ulex. *ACTH significantly lower than tumours that were negatively (NEG) staining and gonadotrophin staining tumours, $P<0.05$. **GH staining tumours significantly more vascular than GH/PRL staining tumours, $P=0.036$.

Figure 3 Vascular density in different types of growth hormone secreting tumours. UEAI: UEAI staining – mean Chalkley counts. GH: growth hormone immuno-positive tumours ($n=14$); GH/PRL: growth hormone and prolactin immuno-positive tumours ($n=11$). Asterisks indicate mean values; error bars indicate the standard error of the mean.

Figure 4 Vascular density in macroprolactinomas. UEAI: UEAI staining – mean Chalkley counts. Non-Inv: non-invasive macroprolactinomas ($n=7$); Inv: invasive macroprolactinomas ($n=8$). Asterisks indicate mean values; error bars indicate the standard error of the mean.

vascular type of functionless tumour using all vascular markers, although this only achieved statistical significance with CD31 ($P<0.05$).

Tumour invasiveness

This was defined according to the modified Hardy criteria (Bates *et al.* 1997). Invasive macroprolactinomas ($n=8$)

were significantly more vascular than non-invasive macroprolactinomas ($n=7$) (Fig. 4) ($P<0.05$ ANOVA, $P=0.042$ *t*-test). The vascular density of invasive macroprolactinomas reached that of the normal pituitary gland. There was no such difference in vascular density between invasive ($n=7$) and non-invasive ($n=21$) GH secreting macroadenomas. Although invasive functionless tumours tended to be more vascular, the differences did not reach statistical significance.

Table 4 Effect of treatment on vascular density. Results are means (S.E.M.). Numbers of cases are in square brackets

	CD31M	CD31G	F8M	F8G	UEAIM	UEAIG
Metyrapone	3.0 (0.0) [2]		4.8 (0.6) [4]	2.0 (0.7) [4]	5.1 (1.7) [6]	2.3 (0.3) [6]
No metyrapone	3.5 (0.0) [1]		4.8 (1.0) [8]	2.0 (0.4) [9]	4.9 (0.7) [7]	2.1 (0.1) [7]
DA Ag Res	6.2 (0.8) [4]	3.5 (0.5) [6]	4.7 (1.2) [9]	2.3 (0.4) [9]	7.5 (0.5) [9]	2.8 (0.2) [9]
DA Ag sensitive	6.4 (0.2) [2]	3.0 (0.0) [2]	5.4 (0.9) [5]	2.4 (0.4) [5]	6.0 (2.0) [4]	2.4 (1.0) [4]
Cushing's cure	3.0 (0.4) [2]		4.6 (0.9) [11]	1.9 (0.3) [13]	4.3 (0.8) [8]*	2.1 (0.1) [8]
No cure	3.3 (0.0) [2]		5.0 (0.9) [3]	1.7 (0.6) [5]	6.0 (1.3) [6]	2.4 (0.3) [7]
Macroprolactinoma cure	6.3 (0.0) [1]	3.0 (0.0) [1]	4.4 (1.6) [2]	2.0 (0.0) [2]	4.8 (3.0) [2]*	1.8 (1.6) [2]
No cure	6.3 (0.5) [8]	3.3 (0.4) [10]	5.0 (0.5) [17]	2.4 (0.3) [17]	7.4 (0.6) [15]*	2.8 (0.2) [16]
GH cure	5.4 (0.5) [13]	2.4 (0.4) [13]	4.7 (0.6) [22]	1.9 (0.3) [24]	7.4 (0.5) [9]	2.7 (0.6) [14]
No cure	5.9 (1.0) [9]	2.5 (0.5) [10]	5.4 (1.2) [11]	2.0 (0.5) [14]	8.1 (0.6) [10]	3.2 (0.5) [11]

DA Ag Res, prolactinomas removed from patients who were resistant to treatment with dopamine agonists; DA Ag sensitive, tumours that were removed from patients who were sensitive to dopamine agonists; GH, GH secreting tumours causing acromegaly; CD31M, mean Chalkley count using endothelial marker CD31; CD31G, semiquantitative grade using endothelial marker CD31; F8M, mean Chalkley count using endothelial marker factor eight-related antigen; F8G, semiquantitative grade using endothelial marker factor eight-related antigen; UEAIM, mean Chalkley count using endothelial marker Ulex; UEAIG, semiquantitative grade using endothelial marker Ulex.

* $P < 0.05$ for difference between cure and no cure.

Response to treatment

Macroprolactinomas and tumours causing Cushing's disease that were cured by trans-sphenoidal surgery were significantly less vascular than those not cured by surgery (Table 4). There was no such association seen with GH secreting tumours.

Tumours removed from patients with Cushing's disease who were treated with metyrapone were of a similar vascular density to tumours in untreated patients (Table 4). There was no difference in MVD in prolactinomas removed from patients who had received treatment with bromocriptine (Table 4). Insufficient numbers of patients with acromegaly received somatostatin agonist treatment prior to surgery for any conclusions to be reached about the effect of octreotide on vascular density.

Comparison of different vascular markers

A comparison (Spearman rank correlation) of the three vascular markers showed that the grade and Chalkley mean using each marker – CD31, UEAI and F8 – were significantly related ($R^2 = 82\%$, $R^2 = 92\%$, and $R^2 = 90\%$ respectively). Comparisons between the three markers showed that CD31 Chalkley mean and grade were significantly related to UEAI Chalkley mean and grade ($P < 0.02$, $R = 0.31$). However the Chalkley mean and grade measured using F8 were poorly related to the measurement using CD31 and UEAI ($P > 0.05$). The total counts using F8 were lower than those using CD31 and UEAI.

Discussion

Vascular counts determined using immunostaining for CD31 and UEAI were more sensitive than F8 and led to

higher overall MVD. Although antibodies to CD31 are not completely specific for endothelial cells, as they may also detect plasma cells, this did not present any practical difficulties (Albelda *et al.* 1991). Ulex is a lectin that binds to fucose containing residues present on endothelial cells, and in some pituitary specimens ulex also stained the Golgi region, making vascular counting impossible (Witt & Klessen 1987). All vascular endothelia do not appear to synthesise factor 8 related antigen (FVIIIa) and so this marker does not stain all microvessels (Mukai *et al.* 1980). This may be related to differences in synthesis of F8. The good correlation shown between counts using CD31 and UEAI corresponds to the fact that these markers have been shown to stain the majority of tumour microvessels, despite the slight drawbacks mentioned above. The poor correlation between both these markers and F8 is in keeping with the more limited expression of this marker. However, despite these differences the results with each marker were similar although the total vascular counts differed. For future studies in the pituitary gland, either UEAI or CD31 are probably optimal, although differences in tissue preservation can make CD31 less straightforward to use.

Tumour microvascular density was clearly related to the age of the patient when tumours secreting GH were considered, fitting with the clinical observation that acromegaly is a milder disease with smaller tumours and lower GH levels in patients who present when older, and it is the younger patients with acromegaly who are likely to have the aggressive larger tumours (Klijn *et al.* 1980, Smals *et al.* 1988). This relationship between angiogenesis and age in GH secreting tumours is not related to size, as we have previously shown that the MVD of microadenomas and macroadenomas secreting GH is similar (Turner *et al.* 2000a), and also the relationship persisted when microadenomas and macroadenomas were analysed

separately. It suggests that there may be a difference in angiogenic mechanisms in pituitary tumours from older patients. There are some data to suggest that angiogenesis is impaired with ageing (Rivad *et al.* 1999). Endothelial cells may become less responsive with age and vascular endothelial growth factor (VEGF) expression is reduced. There was no such statistically significant relationship observed between age and angiogenesis in other tumour types, although there was a trend in all tumour types for the adenomas removed from older patients to have lower MVD. This is in keeping with the observation that there is a trend to lower proliferation indices (measured using Ki-67) in pituitary tumours removed from older patients in comparison with younger patients (Yonezawa *et al.* 1997).

The association between vascular density and pre-treatment prolactin concentration is related to the fact that macroprolactinomas are significantly more vascular than microprolactinomas (Turner *et al.*, 2000a) and higher pre-treatment prolactin concentrations are found in cases with macroprolactinomas. Pre-treatment prolactin concentrations were related to angiogenesis when macroprolactinomas alone were considered. It is interesting to note that although the difference did not reach statistical significance, the vascular density of ACTH producing tumours associated with higher UFC and ACTH were consistently higher than the tumours associated with lower hormonal activity. This is in contrast to GH producing tumours where there is no relationship between tumour size and GH production, and we have shown that there is no relationship of vascular density with tumour size or hormonal production.

Tumours producing GH and prolactin were less vascular than the tumours producing GH alone. It is relevant to speculate whether tumour production of 21 kDa prolactin also reflects production of 16 kDa prolactin – a known inhibitor of angiogenesis (Clapp *et al.* 1993) and that this leads to a restraining influence on angiogenesis in these tumours. The low vascular density of the silent ACTH tumours is difficult to explain when their clinical behaviour is often aggressive. It suggests that there are other factors determining their behaviour, such as alterations in cell cycle control secondary to, for example, alterations in p27 expression (Lidhar *et al.* 1998). The results in functionless tumours are in contrast to those of Pawlikowski *et al.* (1997) who showed that tumour expression of follicle-stimulating hormone was associated with the highest vascular density; however, the total number of tumours studied in their paper was small ($n=22$) and they did not study tumour hot spots (Pawlikowski *et al.* 1997). The differences were only present when vascular density using F8 and ulex were assessed.

A relationship between angiogenesis and tumour behaviour was demonstrated when invasiveness was considered. This was particularly apparent when invasive macroprolactinomas were considered, in keeping with the fact that these tumours tend to be the most aggressively invasive

type of benign pituitary adenoma. Jugenburg and colleagues (1995) showed no difference between invasive and non-invasive tumours in terms of vascular density, although they did demonstrate that if the MVD of hot spots in pituitary carcinomas were assessed, the MVD was higher than in benign adenomas, suggesting an association between tumour behaviour and aggressiveness (Jugenburg *et al.* 1995). There is a biological problem in assessing invasiveness, as associations between tumour behaviour and invasiveness differ depending on the way that invasiveness is assessed – varying from microscopic examination of the dura to intra-operative observation or radiological assessment (Turner & Wass 1999). Invasiveness of pituitary tumours has been shown in other studies to correlate with factors such as tumour proliferation index (Turner & Wass 1999).

There was a significant relationship between angiogenesis and surgical cure in macroprolactinomas and ACTH secreting tumours, as those that were less vascular were more likely to be cured. This is likely to be related to differences in tumour invasiveness and difficulty in completely resecting more vascular tumours. Cure in functionless tumours is difficult to define as there is no hormonal product to use as a tumour marker, but we have shown that MVD cannot be used to predict which functionless tumours will regrow and recur (Turner *et al.*, 2000b).

In Jugenburg's study, prolactinomas removed from patients treated with bromocriptine showed a trend to lower MVD than those removed from untreated patients, although these differences did not achieve statistical significance (Jugenburg *et al.* 1995). In our cohort of tumours, treatment of patients with ACTH secreting tumours with metyrapone, or those with prolactinomas with bromocriptine did not appear to be associated with altered vascular density. This is perhaps not surprising as these drugs are not known to inhibit angiogenesis *per se*. There are significant methodological problems with investigating the effects of these drugs as duration of treatment and compliance are difficult to assess. Finally, the results may be confounded by the administration of these medications to reduce hormonal levels because the tumours in these particular cases were more active and the associated clinical features more florid.

In conclusion, these results show that factors related to angiogenesis (measured using MVD) are very important in determining a number of known clinical features of pituitary tumours, in particular the invasiveness of macroprolactinomas, the effect of age in tumours secreting GH and the outcome of surgical treatment in macroprolactinomas and ACTH secreting tumours. It is possible that inhibitors of angiogenesis may be of value in recurrent prolactinomas and since radiotherapy is more effective in well-oxygenated tumours, that this mode of treatment should be used in more highly angiogenic cases. The relationship between angiogenesis and tumour invasiveness suggests, as shown in other tumour types, that

angiogenesis increases in pituitary adenomas as they become more aggressive. It will be interesting to study MVD in the very rare cases of pituitary carcinoma to determine whether these tumours have an even higher MVD.

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Received 11 October 1999

Accepted 30 December 1999