CLINICAL STUDY

Transforming growth factor β 1 is not a reliable biomarker for valvular fibrosis but could be a potential serum marker for invasiveness of prolactinomas (pilot study)

Atanaska Elenkova, Iliana Atanassova, Georgi Kirilov, Vladimir Vasilev, Krassimir Kalinov¹ and Sabina Zacharieva
Clinical Centre of Endocrinology, USHATE 'Acad. Ivan Pentchev', Medical University, 2 Zdrave Street, Sofia, Bulgaria and ¹Department of Informatics, New Bulgarian University, Sofia, Bulgaria

(Correspondence should be addressed to A Elenkova; Email: atanaskae@yahoo.com)

Abstract

Background: Transforming growth factor β 1 (TGF β 1) signaling pathway is crucial for both human fibrogenesis and tumorigenesis.

Objective: This study aimed to investigate the usefulness of TGF β 1 and matrix metalloproteinase 2 (MMP2) as potential circulating markers for fibrotic valvular heart disease (FVHD) and invasiveness as well as of Fetuin A as a marker for calcification in patients with prolactinomas.

Design: The study population consisted of 147 subjects divided into four groups: 30 dopamine agonist (DA)-treated prolactinoma patients with proven FVHD and three control groups with normal echocardiograms: 43 DA-treated patients, 26 naïve patients, and 48 healthy subjects.

Results: We observed significantly higher serum TGF β 1 levels in all three patient groups than in the healthy subjects (21.4 ± 8.86 vs 19.1 ± 9.03 vs 20.7 ± 11.5 vs 15.8 ± 7.2 ng/ml; $P=0.032$). Moreover, TGF β 1 levels were significantly higher in patients with macroprolactinomas and invasive prolactinomas than in those with microprolactinomas and noninvasive tumors respectively. In addition, a strong positive linear relationship between TGF β 1 levels and invasiveness score ($\rho=0.924$; $P<0.001$) and a moderate correlation between TGF β 1 levels and tumor volume ($r=0.546$; $P<0.002$) were observed in patients with invasive prolactinomas. By contrast, prolactin (PRL) levels exhibited a better correlation with tumor volume ($r=0.721$; $P<0.001$) than with invasiveness score ($\rho=0.436$; $P<0.020$). No significant difference was observed in Fetuin A levels between patients with FVHD and healthy controls. Results concerning MMP2 were unclear.

Conclusions: TGF β 1, MMP2, and Fetuin A are not reliable biomarkers for valvular fibrosis and calcification in DA-treated patients with prolactinomas, but TGF β 1 may represent a useful serum marker for tumor invasiveness. The simultaneous determination of TGF β 1 and PRL levels could improve the noninvasive assessment of prolactinoma behavior.

European Journal of Endocrinology 169 299–306

Introduction

Long-term treatment of Parkinson's disease with the dopamine agonists (DAs) pergolide and cabergoline has been established to be associated with a significantly increased risk of developing fibrotic valvular heart disease (FVHD) (1, 2, 3, 4). The process is triggered by the activation of the serotonin receptors (5HT_{2b}), which are highly expressed in cardiac valves (5, 6, 7). Cabergoline, which possesses a complete 5HT_{2b} agonistic activity, is the drug of choice in the treatment of prolactinomas, but the results of observational studies investigating the risk of FVHD in these patients are still controversial. Some of them have reported no relevant findings (8, 9, 10, 11, 12), five trials have observed clinically insignificant valvular changes (13, 14, 15, 16, 17), and only one study has reported an increased

prevalence of moderate tricuspid regurgitation with a cumulative dose-dependent risk (18). Published data on the potential profibrotic effect of bromocriptine, a partial 5HT_{2b} agonist, are exclusively limited (19). Although there are numerous case reports in the literature, only a few studies have been dedicated to this topic (13, 17, 20, 21). As drug-induced valvulopathy in Parkinsonian patients has been shown to be a cumulative dose-dependent process, for potentially at-risk groups, not only patients receiving high daily doses, but also those on low-dose long-term treatment, such as patients with invasive macroprolactinomas who are often on lifelong therapy, should be considered. In this context, the discovery of reliable circulating invasive markers for fibrosis would help in the early identification of asymptomatic subjects at an increased risk of developing FVHD.

Transforming growth factor β 1 (TGF β 1 (TGFB1)) signaling pathway is crucial for both human fibrogenesis and tumorigenesis (22, 23, 24). TGF β 1 is a profibrotic cytokine with a strong stimulatory effect on the synthesis of collagen and differentiation of cardiac fibroblasts into myofibroblasts, the main function of which is the overproduction of matrix proteins (25, 26, 27, 28). The final outcomes are fibrosis, thickness, and calcification of the valvular cusps and eventually valvular regurgitation. Fetuin A is a glycoprotein acting as a potent inhibitor of the endogenous calcification process, the circulating levels of which have been shown to be decreased in patients with rheumatic valvular lesions, diabetes, and coronary disease, with calcifications of the mitral and aortic valves in comparison with healthy subjects (29, 30, 31). To date, there are no published data on the circulatory levels of TGF β 1 and Fetuin A in subjects with drug-induced fibrotic valvulopathy.

In tumorigenesis, TGF β 1 exerts both tumor suppressor (in the early stage of premalignant cells) and tumor promoter (enhancer of tumor progression, invasion, and metastasis in advanced stages of carcinogenesis) actions (23). Overexpression of TGF β 1 has been demonstrated in many human tumors (e.g. melanoma, breast, prostatic, renal, gastrointestinal, ovarian, cervical, hematological, and brain tumors), in some of which circulatory levels of TGF β 1 have been shown to correlate with poor prognosis (32, 33, 34, 35). Recent studies have supported the critical role of activin/TGF β signaling pathways in pituitary tumorigenesis and, particularly, in prolactinoma formation, although the underlying molecular mechanisms remain to be resolved completely (36, 37).

Subjects and methods

Study design

This observational case-control study was conducted after obtaining approval from the Committee on Research Ethics at the Medical University, Sofia. Eligible subjects were recruited from the participants of our previous study investigating the prevalence of FVHD in patients on long-term cabergoline or bromocriptine treatment (17). From 338 subjects who participated in that study, 147 were enrolled. The high dropout rate was a result of the strict additional exclusion criteria introduced in order to avoid the possible influence of some factors on the circulatory levels of the investigated markers. The primary objective was to compare the circulating levels of TGF β 1, matrix metalloproteinase 2 (MMP2), and Fetuin A as markers for fibrosis and/or calcification between DA-treated prolactinoma patients with proven FVHD (group A; $n=30$) and three control groups with normal echocardiograms: DA-treated patients (group B; $n=43$), medically naïve patients

(group C; $n=26$), and healthy subjects (group D; $n=48$). The secondary objective was to investigate the possible relationship between TGF β 1 and MMP2 levels and the invasiveness of prolactin (PRL)-secreting tumors.

Inclusion criteria

The inclusion criteria of the study were as follows: for groups A and B, patients with prolactinomas treated with one DA (cabergoline or bromocriptine) for at least 12 months; for group C, medically naïve patients with prolactinomas; and for group D, healthy controls frequency matched to groups A and B for age, sex, and obesity.

Exclusion criteria

The exclusion criteria of the study were as follows: for all groups: i) cardiovascular diseases: arterial hypertension; ischemic heart disease; and history of myocarditis, pericarditis, and any previous or concomitant cardiac disease with a substantial risk of heart damage; ii) pulmonary diseases: chronic obstructive pulmonary disease, asthma bronchiale, etc.; iii) hepatic diseases: chronic viral and autoimmune hepatitis, cirrhosis, etc.; iv) systemic connective tissue diseases; v) extracardiac fibrosis (pleuropulmonary, retroperitoneal, hepatic, etc.); vi) use of drugs with potential profibrotic effects on heart valves; vii) diabetes mellitus; viii) documented or suspected inflammatory disease (elevated CRP levels and erythrocyte sedimentation rate, etc.); ix) stroke or ischemic brain disease; x) neoplasms; xi) thrombocytosis; and xii) pregnancy; for groups B, C, and D: valvular fibrosis and regurgitation of any degree.

Study procedures

Study procedures were carried out after the participants and study investigators signed a specific informed consent.

Assay methods

Serum samples were collected at the time of echocardiographic assessment and stored frozen at -80°C until analysis. The measurement of serum TGF β 1, MMP2, and Fetuin A concentrations was based on standard sandwich ELISA using commercial kits of BioVendor (Brno Czech Republic) with analytical sensitivities of 0.0085, <0.05 , and 0.104 ng/ml; intra-assay coefficients of variation (CV) of 3.2, 1.9, and 3.6%; and inter-assay CV of 4.9, <10 , and 6.3% for each of the above-mentioned kits respectively. Serum TGF β 1 and MMP2 levels as potential markers for fibrosis were determined in all four study groups, whereas evaluation of Fetuin A as a factor of calcification was carried out

only in patients with fibrotic valvulopathy compared with healthy controls.

Data on patients' history and anthropometric and echocardiographic parameters were obtained from the database of the above-mentioned study. FVHD was defined as fibrosis (with or without calcification) accompanied or not accompanied by regurgitation. Fibrosis was considered present in all cases with increased echogenicity (hyperechogenicity) and thickness of valvular cusps (> 3 mm for the mitral valve and > 2 mm for the aortic, tricuspid, and pulmonary valves).

Tumor volume was calculated using the formula for an ellipsoid approximation: $\pi/6 \times (X \times Y \times Z)$, where X, Y, and Z are the anteroposterior, vertical, and transverse diameters of the pituitary adenomas on magnetic resonance imaging (MRI). Tumor invasiveness was assessed using the staging system of Sarlis *et al.* (38) based on MRI (Table 1). Cumulative score calculated for each tumor was equal to the sum of three scores: of cavernous and sphenoidal sinus invasion and suprasellar extension.

Statistical analysis

Statistical analysis was carried out using SPSS 17.0 for Windows. Normality of data distribution was assessed using the Kolmogorov–Smirnov test. Metric variables with a Gaussian distribution were described by arithmetic means and s.d. Medians and interquartile ranges were used when data distribution was not normal. Nonmetric variables are presented using relative frequency distribution (%). Testing for equality of means was performed using one-way ANOVA in the case of more than two groups. Multiple *post hoc* comparisons were made according to the Student–Newman–Keuls approach. Student's *t*-test was used when comparing two groups. The hypotheses about nonmetric variables were tested using χ^2 test. The Kruskal–Wallis and Mann–Whitney *U* nonparametric tests were used for variables with a non-Gaussian distribution. Pearson's correlation coefficient *r* and η

coefficient were used to determine the relationships between variables. Spearman's correlation coefficient after ranking of TGFβ1 values was applied to investigate the relationship between TGFβ1 levels and tumor invasiveness. The null hypothesis (of no difference) was rejected if the observed significance (*P* value) was <0.05 (significance level).

Results

The main clinical characteristics of the study participants are summarized in Table 2. The investigated groups did not differ by sex distribution, body surface area, and systolic and diastolic blood pressure. All three patient groups had comparable percentages of subjects with invasive prolactinomas, but significantly differed with respect to their tumor volume and PRL levels at the start of the study. Newly diagnosed patients were significantly younger and had higher PRL levels and larger tumors. Cumulative doses of both cabergoline and bromocriptine were nonsignificantly higher in patients with FVHD than in those with intact valves.

The main echocardiographic parameters are given in Table 3. The Student–Newman–Keuls *post hoc* analysis revealed a significantly higher left ventricular mass (index) in patients with fibrotic valvulopathy than in the healthy subjects (104.1 ± 31.4 vs 97.7 ± 20.7 vs 92.6 ± 18.6 vs 89.6 ± 20.9 ; *P*=0.043). No differences were found in the other echocardiographic parameters. According to the study protocol, all patients in group A had echocardiographically approved valvular fibrosis (74% with monovalvular, 13% with bivalvular, and 13% with trivalvular involvement respectively) accompanied by pathological regurgitations in 30% of the cases. None of the study participants in the other groups had valvular damage and regurgitation of any degree.

Laboratory data are presented in Table 4. We observed significantly higher serum TGFβ1 levels in all three patient groups than in the healthy controls (21.4 ± 8.86 vs 19.1 ± 9.03 vs 20.7 ± 11.5 vs 15.8 ± 7.2 ng/ml; *P*=0.032). MMP2 levels in *de novo* patients

Table 1 Staging system for the assessment of the anatomic relationship of pituitary adenomas with their surrounding structures (38).

Anatomic structures	Grades	Points
Cavernous sinus invasion	Grade 0: no involvement of internal carotid artery circumference	0
	Grade 1: tumor abutting <50% of ICA circumference	1
	Grade 2: tumor abutting ≥50% of ICA circumference	2
	Grade 3: tumor extending into the middle cranial fossa with compression of the temporal lobe	3
Sphenoidal sinus invasion	Grade 0: no erosion or remodeling of the sellar floor	0
	Grade 1: minimal erosion and remodeling of the sellar floor by the tumor	1
	Grade 2: tumor extending into the sphenoidal sinus, occupying <50% of its height	2
	Grade 3: tumor extending into the sphenoidal sinus, occupying ≥50% of its height	3
Suprasellar extension	Grade 0: tumor superior border at or below the diaphragma sellae	0
	Grade 1: tumor extending above the diaphragma sellae, but not abutting the optic chiasm	1
	Grade 2: tumor abutting, but not displacing, the optic chiasm	2
	Grade 3: tumor displacing and compressing the optic chiasm	3

ICA, internal carotid artery.

Table 2 Main characteristics of the study groups.

	DA-treated patients with fibrosis (n=30)	Control groups (with normal echocardiograms)			P ^a
		DA-treated patients (n=43)	Medically naïve patients (n=26)	Healthy controls (n=48)	
Males (% (n))	26.7 (8)	25.6 (11)	26.9 (7)	25.0 (12)	0.997
Age (years); mean ± s.d.	39.3 ± 7.58*	36.0 ± 8.18	32.5 ± 8.14 [†]	36.0 ± 7.55	0.018
BSA (m ²); mean ± s.d.	1.87 ± 0.23	1.84 ± 0.26	1.83 ± 0.29	1.81 ± 0.20	0.708
SBP (mmHg); mean ± s.d.	118.5 ± 7.44	117.2 ± 8.61	119.0 ± 7.21	118.3 ± 7.96	0.803
DBP (mmHg); mean ± s.d.	75.76 ± 6.65	74.7 ± 6.76	75.8 ± 5.78	73.6 ± 4.70	0.391
Invasive (% (n))	26.7 (8)	27.9 (12)	26.9 (7)	–	0.992
TD (months); mean ± s.d.	49.9 ± 33.97	44.0 ± 26.48	–	–	0.399
CD (mg) cabergoline ^b ; mean (range)	171.2 (34–480)	167.8 (24–392)	–	–	0.922
CD (g) bromocriptine ^c ; mean (range)	14.1 (0.9–45.0)	8.37 (0.8–21.6)	–	–	0.335
PRL at study entry (mIU/l); median (IQR)	569.0 [†] (173–1120)	336.0 [†] (162–753)	2520.0* (1776–5226)	328.5 [†] (253–441)	<0.001
PRL at diagnosis (mIU/l); median (IQR)	3002.0 (2078–4800)	3854.5 (2472.5–5331)	3024.0 (1784.5–5589)	–	0.509
Tumor volume (mm ³); median (IQR)	78.0 [†] (29.6–377.8)	52.0 [†] (24.2–232.9)	225.8* (49.4–1733.9)	–	0.023

^aOne-way ANOVA followed by the Student–Newman–Keuls *post hoc* tests (*, [†]significant difference of $P < 0.05$).

^bCabergoline-treated patients: 20 patients with valvular fibrosis and 35 patients without fibrosis.

^cBromocriptine-treated patients: ten patients with FVHD and eight patients without fibrosis.

were lower than those in the DA-treated patients with valvulopathy (2.48 ± 1.23 vs 3.73 ± 1.78 vs 2.97 ± 1.51 vs 2.99 ± 1.24 ng/ml; $P = 0.017$). By contrast, no significant difference was observed in serum Fetuin A levels between patients with valvular fibrosis and healthy subjects (468.2 ± 222.4 vs 494.8 ± 198.8 mkg/ml; $P = 0.606$).

The second part of the study included two main analyses: comparisons of TGFβ1 and MMP2 levels in patients with macroprolactinomas ($n = 28$) vs microprolactinomas ($n = 71$) and invasive ($n = 29$) vs non-invasive ($n = 70$) tumors. Invasive macroprolactinomas were approximately ten times more prevalent than invasive microprolactinomas (82.1 vs 8.45%). Serum

TGFβ1 levels were significantly higher in patients with macroprolactinomas and invasive prolactinomas than in those with microprolactinomas and noninvasive tumors respectively. We found controversial results concerning MMP2 concentrations: a highly significant difference between patients with invasive and non-invasive tumors and comparable levels between those with microprolactinomas and macroprolactinomas (Table 5). Moreover, there was no correlation between serum MMP2 levels and invasiveness score (Pearson's coefficient $r = -0.249$; $P = 0.251$). By contrast, we observed a strong positive linear relationship between TGFβ1 levels and invasiveness score (Spearman's coefficient $\rho = 0.924$; $P < 0.001$) and a medium positive

Table 3 Echocardiographic parameters of the investigated groups (data are expressed as mean ± s.d.).

	DA-treated patients with valvular fibrosis (n=30)	Control groups (with normal echocardiograms)			One-way ANOVA P ^a
		DA-treated patients (n=43)	Medically naïve patients (n=26)	Healthy controls (n=48)	
LVIDS (mm)	28.4 ± 5.72	27.7 ± 4.18	27.7 ± 4.17	27.5 ± 6.17	0.886
IVSTS (mm)	13.5 ± 1.79	13.4 ± 2.34	13.5 ± 1.42	13.3 ± 1.36	0.918
PWTS (mm)	14.9 ± 2.39	14.3 ± 2.37	13.8 ± 1.88	14.2 ± 2.34	0.388
LAI (mm/m ²)	18.5 ± 2.19	18.0 ± 2.18	18.1 ± 2.34	17.7 ± 1.82	0.499
AOi (mm/m ²)	16.4 ± 2.63	16.7 ± 2.29	16.3 ± 2.81	16.4 ± 2.34	0.721
SVi (ml/m ²)	37.9 ± 11.9	34.8 ± 8.03	33.2 ± 8.54	36.0 ± 8.95	0.275
LVMi (g/m ²)	104.1 ± 31.4*	97.7 ± 20.73	92.6 ± 18.6	89.6 ± 20.9 [†]	0.043
Valvular fibrosis (% (n))	100 (30)	0	0	0	<0.001
Pathological regurgitations (% (n))	30 (9)	0	0	0	<0.001

LVIDS, left ventricular internal dimension in systole; IVSTS, interventricular septum thickness systole; PWTS, posterior wall thickness systole; LAI, left atrium index; AOi, maximum aortic diameter index; LVMi, left ventricular mass (index); SVi, stroke volume (index).

^aOne-way ANOVA followed by the Student–Newman–Keuls *post hoc* tests (*, [†]significant difference of $P < 0.05$).

Table 4 Serum TGFβ1, MMP2, and Fetuin A levels.

	DA-treated patients with fibrosis (n=30)	DA-treated patients without fibrosis (n=43)	Medically naïve patients (n=26)	Healthy controls (n=48)	P ^a
TGFβ1 (ng/ml)	21.4±8.86*	19.1±9.03*	20.7±11.5*	15.8±7.2†	0.032
MMP2 (ng/ml)	3.73±1.78*	2.97±1.51	2.48±1.23†	2.99±1.24	0.017
Fetuin A (mkg/ml)	468.2±222.4	—	—	494.8±198.8	0.606

^aOne-way ANOVA followed by the Student–Newman–Keuls *post hoc* tests (*†significant difference of $P<0.05$).

linear correlation between TGFβ1 levels and tumor volume (Pearson's coefficient $r=0.546$; $P=0.002$). As the investigated groups differed with respect to their sex ratio, the role of gender as a potential confounding factor was investigated. We did not find a significant difference in TGFβ1 levels between male and female healthy controls (13.8 ± 9.29 vs 16.5 ± 6.38 ; $P=0.358$). In addition, based on the bivariate correlation analysis, we did not find a relationship between TGFβ1 levels and gender either among the healthy subjects (Pearson's coefficient $r=-0.166$; $P=0.259$) or among the patients (Pearson's coefficient $r=0.142$; $P=0.161$). A lack of a relationship between TGFβ1 levels and age was also observed in the healthy subjects (Pearson's coefficient $r=-0.124$; $P=0.4$) as well as in prolactinoma patients (Pearson's coefficient $r=0.074$; $P=0.467$). No significant difference was found in TGFβ1 levels between DA-treated ($n=73$) and naïve ($n=26$) patients (19.76 ± 9.21 vs 20.73 ± 11.55 ng/ml; $P=0.069$).

The relationships between PRL and TGFβ1 levels and tumor volume and invasiveness in patients with invasive tumors were investigated using bivariate correlation. PRL levels exhibited a better correlation with tumor volume ($r=0.721$; $P<0.001$) than with invasiveness score ($\rho=0.436$; $P<0.020$), whereas TGFβ1 levels seemed to exhibit a stronger correlation with tumor invasiveness ($\rho=0.924$; $P<0.001$) than with tumor volume ($r=0.546$; $P=0.002$). The linear regression model was adequate ($F=589.3$; $P<0.001$) and revealed a strong positive linear correlation between both markers and tumor invasiveness ($r=0.989$; $r^2=0.978$; adjusted $r^2=0.977$).

Discussion

TGFβ1 is a key mediator in the pathophysiology of both tissue fibrosis and tumorigenesis. Drug-induced activation of 5HT_{2b} receptors in cardiac valves leads to the dissociation of the heterotrimeric G-protein, the Gαq subunit of which activates phospholipase C, protein kinase C cascade, steroid receptor coactivator, and ERKs, resulting in the upregulation of the expression of TGFβ1 (39, 40). TGFβ1 increases the production of collagen by a dual mechanism: activation of the collagen type I gene promoter via second messengers (SMAD proteins) and stimulation of the translation of other profibrotic factors such as connective tissue growth factor and MMPs, especially MMP2, which facilitates the motility and migration of cardiac fibroblasts (41, 42, 43). A strong negative correlation has been found between the increased local expression of TGFβ1 and its serum levels, which have been shown to be significantly lower in subjects with advanced coronary atherosclerosis and degenerative mitral and aortic regurgitation in comparison with healthy controls (44, 45, 46). A similar negative relationship has also been demonstrated for circulating Fetuin A levels in patients with rheumatic valvular lesions as well as subjects with ischemic heart disease and calcifications of the mitral and aortic valves (30, 31). In contrast to these studies, we did not find any difference in serum Fetuin A levels between long-term DA-treated prolactinoma patients with proven FVHD and healthy controls. Our data concerning MMP2 concentrations were controversial. These results were not unexpected, as fibrosis was limited only to the cardiac valvular apparatus and the percentage of subjects with advanced

Table 5 Comparisons of the levels of the investigated markers: microprolactinomas vs macroprolactinomas and invasive vs noninvasive tumors.

	Tumor size			Invasiveness		
	Macroadenomas (n=28)	Microadenomas (n=71)	P value	Invasive tumors (n=29)	Noninvasive tumors (n=70)	P value
Sex ratio (females:males)	10:18	63:8	<0.0001	11:18	62:8	<0.0001
Age (years); mean±s.d.	33.9±9.23	36.9±7.82	0.132	34.1±8.92	36.9±7.96	0.145
PRL (mIU/ml); median (IQR)	1080 (201–5529)	642 (201.5–1500)	0.072	785.5 (163.3–5313)	710.5 (241.5–1519.5)	0.600
Tumor volume (mm ³); median (IQR)	2687.1 (959.4–7441.2)	41.6 (24.2–109.2)	<0.0001	1684.8 (611–7020)	46.8 (24.96–128.9)	<0.0001
DA-treated patients (% (n))	60.7 (17)	78.9 (56)	0.064	69.0 (20)	74.6 (53)	0.488
TGFβ1 (ng/ml); mean±s.d.	23.9±10.3	18.5±9.25	0.018	24.1±9.9	18.3±9.35	0.010
MMP2 (ng/ml); mean±s.d.	3.30±1.54	3.03±1.61	0.452	3.81±1.29	2.83±1.62	0.003

FVHD in our cohort was extremely low. The analysis of TGFβ1, as opposed to those of MMP2 and Fetuin A, revealed interesting results. On the one side, there was no significant difference in the serum levels between DA-treated patients with FVHD and control groups of DA-treated and medically naïve patients with normal echocardiograms. Moreover, their levels were significantly higher than those in the control group of healthy subjects, although we tried to exclude all factors with possible influence such as atherosclerotic, inflammatory, neoplastic, allergic, and connective tissue diseases; diabetes mellitus; etc. (47, 48, 49, 50). Possible impacts of age and gender as well as DA treatment as confounding factors were also eliminated. These results suggested not fibrosis-associated elevation, but tumor-related elevation in circulating TGFβ1 levels. In support of such a hypothesis were the highly significant difference in serum TGFβ1 concentrations between patients with invasive and noninvasive prolactinomas and the almost perfect positive linear correlation ($\rho = 0.924$; $P < 0.001$) between TGFβ1 levels and the degree of tumor invasion. Markedly higher TGFβ1 levels were also found in patients with macroprolactinomas vs those with microprolactinomas. Nevertheless, the relationship between TGFβ1 levels and tumor volume was found to be positive, but not so strong from a statistical point of view, probably as a result of the errors associated with the use of a geometric approximation model for the calculation of tumor volume. Our results are entirely consistent with the evidence provided by recent *in vitro* studies in favor of the crucial role of TGFβ1 signaling pathway in the regulation of lactotroph cell proliferation, *PRL* gene expression and pituitary tumorigenesis (36, 51, 52). Furthermore, one new immunohistochemical study has demonstrated a significantly higher percentage of TGFβ1 positivity in patients with invasive prolactinomas than in those with noninvasive prolactinomas (53). Although the term 'aggressiveness' should not be used synonymously with 'invasiveness' concerning prolactinomas, almost all aggressive tumors are invasive. Detailed histological subtyping has been established to be the best independent predictor of aggressive behavior, but FGFR4, MMP, PTTG, Ki-67, p53, and deletions in chromosome 1 may serve as additional markers (54). Predominantly conservative management of PRL-secreting adenomas, however, determines the need for reliable noninvasive markers. To the best of our knowledge, this is the first study to provide evidence that TGFβ1 may represent a useful serum marker for invasiveness in patients with prolactinomas. It is important to note that a lot of factors influencing the levels of this cytokine should be excluded before analysis to ensure that its informative value is not reduced. The strict observance of all these exclusion criteria resulted in relatively small sample sizes of the investigated groups, and it represents the main limitation of the study. In spite of the exclusion of all factors with a possible impact on TGFβ1 and use of

appropriate statistical methods, we could not completely eliminate sample selection bias due to the recruitment model designed to correspond best to the primary objective of the study.

Conclusions

In contrast to all other types of hormone-secreting pituitary tumors, for prolactinomas, long-term conservative management with DAs is the treatment of choice and indications for neurosurgery are exclusively restricted. In patients with invasive macroprolactinomas, this treatment is usually lifelong. Consequently, clinicians need reliable noninvasive markers for valvular fibrosis as well as tumor invasiveness, which are two strong determinants of treatment planning and prognosis. Our results demonstrate that TGFβ1 and MMP2 cannot be used as circulating markers for valvular fibrosis and Fetuin A is not a reliable marker for calcification in subjects on long-term DA treatment. On the other hand, TGFβ1 may represent a useful serum marker for invasiveness in patients with prolactinomas. The simultaneous determination of serum TGFβ1 and PRL levels could improve the noninvasive assessment of prolactinoma behavior.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by a grant from the Medical University - Sofia (contract no. 50/2012).

References

- 1 Pritchett AM, Morrison JF, Edwards WD, Schaff HV, Connolly HM & Espinosa RE. Valvular heart disease in patients taking pergolide. *Mayo Clinic Proceedings* 2002 **77** 1280–1286. (doi:10.4065/77.12.1280)
- 2 Van Camp G, Flamez A, Cosyns B, Goldstein J, Perdaens C & Schoors D. Heart valvular disease in patients with Parkinson's disease treated with high-dose pergolide. *Neurology* 2003 **61** 859–861. (doi:10.1212/01.WNL.0000083985.00343.F2)
- 3 Yamamoto M, Uesugi T & Nakayama T. Dopamine agonists and cardiac valvulopathy in Parkinson disease: a case-control study. *Neurology* 2006 **67** 1225–1229. (doi:10.1212/01.wnl.0000238508.68593.1d)
- 4 Zanettini R, Antonini A, Gatto G, Gentile R, Tesei S & Pezzoli G. Valvular heart disease and the use of dopamine agonists for Parkinson's disease. *New England Journal of Medicine* 2007 **356** 39–46. (doi:10.1056/NEJMoa054830)
- 5 Rothman RB, Baumann MH, Savage JE, Rauser L, McBride A, Hufeisen SJ & Roth BL. Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation* 2000 **102** 2836–2841. (doi:10.1161/01.CIR.102.23.2836)

- 6 Kaumann AJ & Levy FO. 5-Hydroxytryptamine receptors in the human cardiovascular system. *Pharmacology & Therapeutics* 2006 **111** 674–706. (doi:10.1016/j.pharmthera.2005.12.004)
- 7 Schade R, Andersohn F, Suissa S, Haverkamp W & Garbe E. Dopamine agonists and the risk of cardiac-valve regurgitation. *New England Journal of Medicine* 2007 **356** 29–38. (doi:10.1056/NEJMoa062222)
- 8 Bogazzi F, Buralli S, Manetti L, Raffielli V, Cigni T, Lombardi M, Boresi F, Taddei S, Salvetti A & Martino E. Treatment with low doses of cabergoline is not associated with increased prevalence of cardiac valve regurgitation in patients with hyperprolactinaemia. *International Journal of Clinical Practice* 2008 **62** 1864–1869. (doi:10.1111/j.1742-1241.2008.01779.x)
- 9 Herring N, Szmigielski C, Becher H, Karavitaki N & Wass JA. Valvular heart disease and the use of cabergoline for the treatment of prolactinoma. *Clinical Endocrinology* 2009 **70** 104–108. (doi:10.1111/j.1365-2265.2008.03458.x)
- 10 Vallette S, Serri K, Rivera J, Santagata P, Delorme S, Garfield N, Kahtani N, Beauregard H, Aris-Jilwan N, Houde G *et al.* Long-term cabergoline therapy is not associated with valvular heart disease in patients with prolactinomas. *Pituitary* 2009 **12** 153–157. (doi:10.1007/s11102-008-0134-2)
- 11 Tan T, Cabrita IZ, Hensman D, Grogono J, Dhillo WS, Baynes KC, Eliahoo J, Meeran K, Robinson S, Nihoyannopoulos P *et al.* Assessment of cardiac valve dysfunction in patients receiving cabergoline treatment for hyperprolactinaemia. *Clinical Endocrinology* 2010 **73** 369–374. (doi:10.1111/j.1365-2265.2010.03827.x)
- 12 Lafeber M, Stades AM, Valk GD, Cramer MJ, Teding van Berkhout F & Zelissen PM. Absence of major fibrotic adverse events in hyperprolactinemic patients treated with cabergoline. *European Journal of Endocrinology* 2010 **162** 667–675. (doi:10.1530/EJE-09-0989)
- 13 Kars M, Delgado V, Holman ER, Feelders RA, Smit JW, Romijn JA, Bax JJ & Pereira AM. Aortic valve calcification and mild tricuspid regurgitation but no clinical heart disease after 8 years of dopamine agonist therapy for prolactinoma. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 3348–3356. (doi:10.1210/jc.2007-2658)
- 14 Lancellotti P, Livadariu E, Markov M, Daly AF, Burlacu MC, Betea D, Pierard L & Beckers A. Cabergoline and the risk of valvular lesions in endocrine disease. *European Journal of Endocrinology* 2008 **159** 1–5. (doi:10.1530/EJE-08-0213)
- 15 Wakil A, Rigby AS, Clark AL, Kallvikbacka-Bennett A & Atkin SL. Low dose cabergoline for hyperprolactinaemia is not associated with clinically significant valvular heart disease. *European Journal of Endocrinology* 2008 **159** R11–R14. (doi:10.1530/EJE-08-0365)
- 16 Nachtigall LB, Valassi E, Lo J, McCarty D, Passeri J, Biller BM, Miller KK, Utz A, Grinspoon S, Lawson EA *et al.* Gender effects on cardiac valvular function in hyperprolactinaemic patients receiving cabergoline: a retrospective study. *Clinical Endocrinology* 2010 **72** 53–58. (doi:10.1111/j.1365-2265.2009.03608.x)
- 17 Elenkova A, Shabani R, Kalinov K & Zacharieva S. Increased prevalence of subclinical cardiac valve fibrosis in patients with prolactinomas on long-term bromocriptine and cabergoline treatment. *European Journal of Endocrinology* 2012 **167** 17–25. (doi:10.1530/EJE-12-0121)
- 18 Colao A, Galderisi M, Di Sarno A, Pardo M, Gaccione M, D'Andrea M, Guerra E, Pivonello R, Lerro G & Lombardi G. Increased prevalence of tricuspid regurgitation in patients with prolactinomas chronically treated with cabergoline. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 3777–3784. (doi:10.1210/jc.2007-1403)
- 19 Jähnichen S, Horowski R & Pertz H. Agonism at 5-HT_{2B} receptors is not a class effect of the ergolines. *European Journal of Pharmacology* 2005 **513** 225–228. (doi:10.1016/j.ejphar.2005.03.010)
- 20 Tan LC, Ng KK, Au WL, Lee RK, Chan YH & Tan NC. Bromocriptine use and the risk of valvular heart disease. *Movement Disorders* 2009 **24** 344–349. (doi:10.1002/mds.22228)
- 21 Boguszewski CL, Dos Santos CM, Sakamoto KS, Marini LC, de Souza AM & Azevedo M. A comparison of cabergoline and bromocriptine on the risk of valvular heart disease in patients with prolactinomas. *Pituitary* 2011 **15** 44–49. (doi:10.1007/s11102-011-0339-7)
- 22 Shi Y & Massagué J. Mechanisms of TGF- β signaling from cell membrane to the nucleus. *Cell* 2003 **113** 685–700. (doi:10.1016/S0092-8674(03)00432-X)
- 23 Massagué J. TGF β in cancer. *Cell* 2008 **134** 215–230. (doi:10.1016/j.cell.2008.07.001)
- 24 Massagué J. TGF β signaling in context. *Nature Reviews. Molecular Cell Biology* 2012 **13** 616–630. (doi:10.1038/nrm3434)
- 25 Sigel AV, Centrella M & Eghbali-Webb M. Regulation of proliferative response of cardiac fibroblasts by transforming growth factor- β 1. *Journal of Molecular and Cellular Cardiology* 1996 **28** 1921–1929. (doi:10.1006/jmcc.1996.0185)
- 26 Souders CA, Bowers SL & Baudino TA. Cardiac fibroblast: the renaissance cell. *Circulation Research* 2009 **105** 1164–1176. (doi:10.1161/CIRCRESAHA.109.209809)
- 27 Khan R & Sheppard R. Fibrosis in heart disease: understanding the role of transforming growth factor- β 1 in cardiomyopathy, valvular disease and arrhythmia. *Immunology* 2006 **118** 10–24. (doi:10.1111/j.1365-2567.2006.02336.x)
- 28 Rabkin E, Aikawa M, Stone JR, Fukumoto Y, Libby P & Schoen FJ. Activated interstitial myofibroblasts express catabolic enzymes and mediate matrix remodeling in myxomatous heart valves. *Circulation* 2001 **104** 2525–2532. (doi:10.1161/hc4601.099489)
- 29 Price PA & Lim JE. The inhibition of calcium phosphate precipitation by fetuin is accompanied by the formation of fetuin–mineral complex. *Journal of Biological Chemistry* 2003 **278** 22144–22152. (doi:10.1074/jbc.M300744200)
- 30 Ix JH, Chertow GM, Shlipak MG, Brandenburg VM, Ketteler M & Whooley MA. Association of fetuin-A with mitral annular calcification and aortic stenosis among persons with coronary heart disease: data from the Heart and Soul Study. *Circulation* 2007 **115** 2533–2539. (doi:10.1161/CIRCULATIONAHA.106.682450)
- 31 Cagli K, Basar N, Cagli K, Armutcu F, Aylik F, Yalcinkaya A, Erden G & Kadirogullari E. Association of serum fetuin-A with valvular calcium concentration in rheumatic mitral valve disease. *Journal of Heart Valve Disease* 2010 **19** 636–643.
- 32 Loh JK, Lieu AS, Su YF, Cheng CY, Tsai TH, Lin CL, Lee KS, Hwang SL, Kwan AL, Wang CJ *et al.* Plasma levels of transforming growth factor- β 1 before and after removal of low- and high-grade astrocytomas. *Cytokine* 2013 **61** 413–418. (doi:10.1016/j.cyto.2012.11.011)
- 33 Schneider T, Sailer M, Ansorge S, Firsching R & Reinhold D. Increased concentrations of transforming growth factor β 1 and β 2 in the plasma of patients with glioblastoma. *Journal of Neuro-Oncology* 2006 **1** 61–65. (doi:10.1007/s11060-005-9116-7)
- 34 Dave H, Shah M, Trivedi S & Shukla S. Prognostic utility of circulating transforming growth factor β 1 in breast cancer patients. *International Journal of Biological Markers* 2012 **27** 53–59. (doi:10.5301/IBM.2011.8736)
- 35 Narai S, Watanabe M, Hasegawa H, Nishibori H, Endo T, Kubota T & Kitajima M. Significance of transforming growth factor β 1 as a new tumor marker for colorectal cancer. *International Journal of Cancer* 2002 **97** 508–511. (doi:10.1002/ijc.1631)
- 36 Lebrun JJ. Activin, TGF- β and menin in pituitary tumorigenesis. *Advances in Experimental Medicine and Biology* 2009 **668** 69–78.
- 37 Kaji H, Canaff L, Lebrun JJ, Goltzman D & Hendy GN. Inactivation of menin, a Smad3-interacting protein, blocks transforming growth factor type β signaling. *PNAS* 2001 **98** 3837–3842. (doi:10.1073/pnas.061358098)
- 38 Sarlis NJ, Gourgiosis L, Koch CA, Skarulis MC, Brucker-Davis F, Doppman JL, Oldfield EH & Patronas NJ. MR imaging features of thyrotropin-secreting pituitary adenomas at initial presentation. *American Journal of Roentgenology* 2003 **181** 577–582. (doi:10.2214/ajr.181.2.1810577)
- 39 Roth BL. Drugs and valvular heart disease. *New England Journal of Medicine* 2007 **356** 6–9. (doi:10.1056/NEJMp068265)

- 40 Jian B, Xu J, Connolly J, Savani RC, Narula N, Liang B & Levy RJ. Serotonin-induced up-regulation of transforming growth factor- β 1 via G-protein signal transduction in aortic valve interstitial cells. *American Journal of Pathology* 2002 **161** 2111–2121. (doi:10.1016/S0002-9440(10)64489-6)
- 41 Greene RM, Nugent P, Mukhopadhyay P, Warner DR & Pisano MM. Intracellular dynamics of Smad-mediated TGF β signaling. *Journal of Cellular Physiology* 2003 **197** 261–271. (doi:10.1002/jcp.10355)
- 42 Moussad EF & Brigstok DR. Connective-tissue growth factor: what's in a name? *Molecular Genetics and Metabolism* 2000 **71** 276–292. (doi:10.1006/mgme.2000.3059)
- 43 Stawowy P, Margeta C, Kallish H, Seidah NG, Chretien M, Fleck E & Graf K. Regulation of matrix metalloproteinase MT1-MMP/MMP-2 in cardiac fibroblasts by TGF- β ₁ involves furin-convertase. *Cardiovascular Research* 2004 **63** 87–97. (doi:10.1016/j.cardiores.2004.03.010)
- 44 Attaran S, Sherwood R, Dastidar MG & El-Gamel A. Identification of low circulatory transforming growth factor β 1 in patients with degenerative heart valve disease. *Interactive Cardiovascular and Thoracic Surgery* 2010 **11** 791–793. (doi:10.1510/icvts.2010.244384)
- 45 Grainger DJ, Kemp PR, Metcalfe JC, Liu AC, Lawn RM, Williams NR, Grace AA, Schofield PM & Chauhan A. The serum concentration of active TGF- β 1 is severely depressed in advanced atherosclerosis. *Nature Medicine* 1995 **1** 74–79. (doi:10.1038/nm0195-74)
- 46 Tashiro H, Shimokawa H, Yamamoto K, Momohara M, Tada H & Takeshita A. Altered plasma levels of cytokines in patients with ischemic heart disease. *Coronary Artery Disease* 1997 **8** 143–147. (doi:10.1097/00019501-199703000-00004)
- 47 Stoll P, Wuertemberger U, Bratke K, Zingler C, Virchow CJ & Lommatzsch M. Stage-dependent association of BDNF and TGF- β 1 with lung function in stable COPD. *Respiratory Research* 2012 **13** 116. (doi:10.1186/1465-9921-13-116)
- 48 Jakuš V, Sapák M & Kostolanská J. Circulating TGF- β 1, glycation, and oxidation in children with diabetes mellitus type 1. *Experimental Diabetes Research* 2012 **2012**. Article ID 510902. (doi:10.1155/2012/510902)
- 49 Joseph J, Benedict S, Badrinath P, Wassef S, Joseph M, Abdulkhalik S & Nicholls MG. Elevation of plasma transforming growth factor β 1 levels in stable nonatopic asthma. *Annals of Allergy, Asthma & Immunology* 2003 **91** 472–476. (doi:10.1016/S1081-1206(10)61516-5)
- 50 Shi M, Yu B, Gao H, Mu J & Ji C. Matrix metalloproteinase 2 overexpression and prognosis in colorectal cancer: a meta-analysis. *Molecular Biology Reports* 2013 **40** 617–623. (doi:10.1007/s11033-012-2100-3)
- 51 Sarkar DK, Chaturvedi K, Oomizu S, Boyadjieva NI & Chen CP. Dopamine, dopamine D2 receptor short isoform, transforming growth factor (TGF)- β 1, and TGF- β type II receptor interact to inhibit the growth of pituitary lactotropes. *Endocrinology* 2005 **146** 4179–4188. (doi:10.1210/en.2005-0430)
- 52 Coya R, Alvarez CV, Perez F, Gianzo C & Diéguez C. Effects of TGF- β 1 on prolactin synthesis and secretion: an *in-vitro* study. *Journal of Neuroendocrinology* 1999 **11** 351–360. (doi:10.1046/j.1365-2826.1999.00336.x)
- 53 Jiang M, Mou CZ, Han T, Wang M & Yang W. Thrombospondin-1 and transforming growth factor- β 1 levels in prolactinoma and their clinical significance. *Journal of International Medical Research* 2012 **40** 1284–1294. (doi:10.1177/147323001204000407)
- 54 Mete O, Ezzat S & Asa SL. Biomarkers of aggressive pituitary adenomas. *Journal of Molecular Endocrinology* 2012 **49** R69–R78. (doi:10.1530/JME-12-0113)

Received 26 January 2013

Revised version received 15 May 2013

Accepted 24 June 2013