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Macroprolactinaemia, the major unknown in the differential diagnosis of hyperprolactinaemia

10 cases and discussion of diagnostic clues

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Summary

We report on 10 cases of macroprolactinaemia and discuss recent evidence that many patients with hyperprolactinaemia (8–26%, depending on the population studied) have in fact normal amounts of circulating prolactin but false-high values in commercial assays. This is caused by macromolecular prolactin (also named big-big prolactin or macroprolactin), a complex of prolactin with IgG antibodies leading to apparent hyperprolactinaemia. In spite of the expanding literature on this topic, it remains an underrecognised problem, typically causing unnecessary procedures such as laboratory controls, MRI of the pituitary, treatment with dopamine agonists or even pituitary surgery. Physicians involved with diagnosis and treatment of hyperprolactinaemia (general practitioners, gynaecologists, neurosurgeons, endocrinologists and biochemists) should suspect the presence of apparent hyperprolactinaemia in any patient with a high prolactin value but no related symptoms. Medical laboratories should be aware that their prolactin assay can interfere with macroprolactin and should implement the use of the PEG precipitation test in the work-up of hyperprolactinaemia, a simple and effective means of correctly diagnosing apparent hyperprolactinaemia.

Keywords: macroprolactinaemia; big-big prolactin; apparent hyperprolactinaemia; idiopathic hyperprolactinaemia

Introduction

In the diagnosis of galactorrhoea, amenorrhoea or other menstrual disorders, and after radiological diagnosis of intrasellar masses, determination of prolactin is indicated. Differential diagnosis of hyperprolactinaemia embraces a wide spectrum. Physiological hyperprolactinaemias are seen in stress and pain states, excessive physical training and pregnancy. The commonest causes of non-physiological hyperprolactinaemia are prolactinoma (prolactin-secreting pituitary adenoma), lesions in the hypothalamo-pituitary region (non-prolactinoma) which interfere with prolactin-inhibiting neurones (typically by compression of the pituitary stalk) and a drug-induced/iatrogenic cause (oestrogen compounds, neuroleptics, antidepressive agents, metoclopramide etc.). Further possible causes are, for example, hypothyroidism, repetitive-mechanical irritation of the nipple, empty sella syndrome and renal failure. A further little known cause of hyperprolactinaemia is macroprolactin. On the basis of clinical examples, the signs indicative of macroprolactinaemia are pointed out; this is followed by discussion of pathogenesis, diagnosis and consequences.

Patients and methods

Between October 1998 and September 2000 10 patients were investigated for macroprolactinaemia in an endocrinological consultancy. Patient data are shown in Table 1. The main reason for the work-up was the discrepancy between elevated prolactin value on the one hand and the clinical findings on the other. Despite significant hyperprolactinaemia, galactorrhoea and persistent amenorrhoea were present in only two cases, erectile function and sexual life were unaffected, pituitary MRI failed to show pituitary masses (with the sole exception of pat. 6, in whom a prolactinoma was found at operation. Although in pat. 1 MRI prompted suspicion of an in-

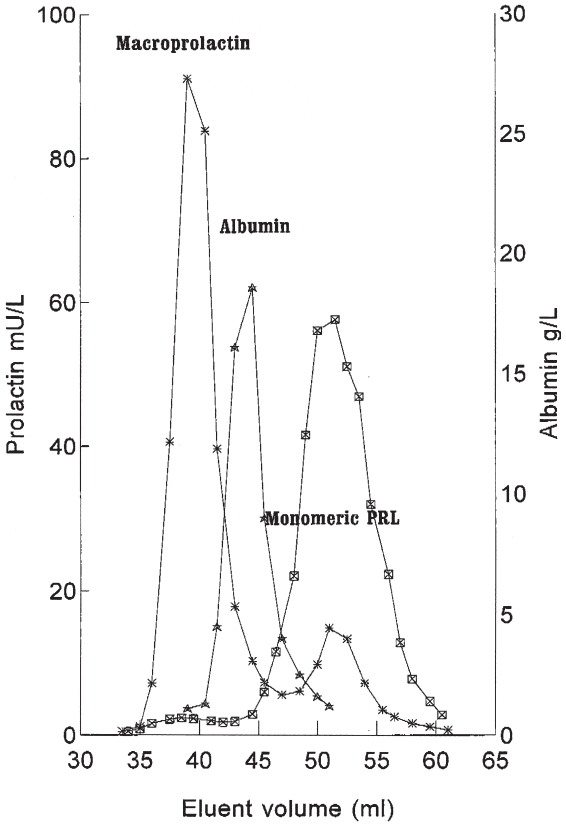

Figure 1

Gel filtration chromatography of serum prolactin in a female patient with macroprolactinaemia (pat. 1, ✳) and a control person (⊠). Albumin peak (☆) as marker for molecular weight.

trasellar mass, surgical exploration failed to demonstrate adenomatous tissue). A further reason for the search for macroprolactinaemia was lack of a clear diagnosis, since in 8 of the 10 cases no cause could be found for the hyperprolactinaemia (idiopathic hyperprolactinaemia). In the macroprolactin investigation a precipitation test with polyethylene glycol (PEG) was performed in all of our patients, as well as gel filtration chromatography in 7 of 10 cases.

Methods

The employed procedures are the same as previously described [1].

Prolactin assay

The analysis was performed by the double antigen technique (solid phase fluoroimmuno-assay, Auto Delfia, Wallac, UK). The normal range for the test used is <19 µg/l. The intra-assay variance of this test has a coefficient of variation (CV) of 3%. The CV for inter-assay variance is 2%.

PEG-precipitation test

200 µl polyethylene glycol (PEG; molecular weight 6000) was mixed with the same volume of serum and centrifuged at 3000 rpm for 30 min. Prolactin was determined before and (in the supernatant) immediately after PEG precipitation. The result was expressed as prolactin recovery, calculated according to the following formula: [immunoreactive prolactin after PEG precipitation / immunoreactive prolactin before PEG precipitation] ×100. Diagnosis of macroprolactinaemia was regarded as certain if the prolactin recovery in a serum was <40% (as described below). In selected cases the macroprolactinaemia diagnosis was also confirmed by gel filtration chromatography (GFC, Sephacryl S-300, Pharmacia). 1 ml serum was applied to a chromatography column and diluted with 10 mmol/l TRIS buffer (pH 7.40, 140 mmol/l NaCl, 1.25 mmol/l CaCl, 0.50 mmol/l MgCl, flow rate 0.5 ml/min). After the first 30 ml of eluent had been discarded 40 fractions of 1 ml were collected and prolactin was then determined in each fraction (Auto Delfia, Wallac, UK). Thus the two prolactin immunoreactivity peaks were visible and macroprolactin was quantified from the area under the curve (Figure 1). Albumin (BCG) was additionally determined from 10 fractions. The position of the albumin peak was used as a chromatographic control.

Discussion

In a considerable number of patients with hyperprolactinaemia none of the causes mentioned above can be demonstrated; the condition is then called idiopathic hyperprolactinaemia. There are also cases where hyperprolactinaemia can be demonstrated by laboratory tests, but without a clinical correlate (i.e. amenorrhoea and galactorrhoea in women; hypogonadism, erectile dysfunction and gynaecomastia-galactorrhoea in men). In such cases the clinician must always raise the question whether true hyperprolactinaemia is present. And indeed we are often confronted – as in our patients – with a merely "apparent hyperprolactinaemia". *How does this come about?*

The glycoprotein prolactin circulates in the blood chiefly as a monomeric molecule weighing 23 kD. Chromatographic investigations have shown that 2 further forms of prolactin exist, called "big prolactin" and "big-big prolactin" (molecular weight 50 and >100 kD respectively). This heterogeneity of prolactin has been documented by several authors since as long ago as 1974 [2–4]. Several years later were anti-PRL autoantibodies detected in patients with "big-big prolactin": these were IgG antibodies with low receptor affinity [5–8]. Such autoantibodies lead to aggregation of prolactin monomers in macromolecular prolactin (macroprolactin or "big-big prolactin"). Macroprolactin is analogous to macroamylase, macro-CK type 1, macro LDH, macro alkaline phosphatase, macro AST and macro ALT, in that all these macro forms are largely analyte-antibody complexes. There is no evidence that one macro form can be found in association with another.

Macroprolactin interferes with commercial immunoassays leading to false-high prolactin values ("apparent hyperprolactinaemia"). A typical feature of this is the varying degree of prolactin elevation depending on the measurement method employed [9]. An example is given by analysis of the same serum sample containing 93% macroprolactin (real value for monomeric prolactin 7.1 µg/l) which, with five different assays, gives results between 26 and 143 µg/l (see Table 2). It is worth noting that 2 of the 3 largest private medical laboratories and 4 of the 5 university laboratories in Switzerland are using at present prolactin assays

Table 1

Clinical data of 10 patients with macroprolactinaemia.

Patient	sex and age[a]	initial clinical findings	PRL (n <20 µg/l)	macroprolactin (recovery, n >50%)	pituitary MRI	treatment	course
1	female, age 37	3-month secondary amenorrhoea, effort-induced	126	positive (9%)	4 mm intrasellar tumour	transsphenoidal exploration[b]	stable hyper-PRL 1 year after surgery; menses normalised
2	female, age 18	polymenorrhoea, galactorrhoea	59	positive (9.3%)	suspected small intrasellar tumour	quinagolide 150 µg/day	2 years later stable hyper-PRL on quinagolide; menses normalised
3	female, age 24	headache, regular menses	245	positive (17%)	normal	quinagolide 75 µg/day	6 months later stable hyper-PRL on quinagolide; menses normalised
4	female, age 28	oligomenorrhoea with PCOS[c]	39	positive (24%)	normal	nihil	7 months later stable hyper-PRL; persistent oligo-menorrhoea
5	male, age 27	infertility hypogonadism	34	positive (8.9%)	normal	bromocriptine 5 mg	remission with 2 spontaneous conceptions by wife; PRL normalised on bromocriptine
6	female, age 32	galactorrhoea, sec. amenorrhoea	68	positive (9.5%)	suspected microadenoma	transsphenoidal exploration: immuno-histo-chemically prolactinoma	development of primary ovarian failure; persistent hyper-PRL and galactorrhoea 6 months post-op.
7	female, age 52	fatigue and headache (post-menopause)	179	positive (2.8%)	normal	nihil	4 years later stable hyper-PRL, asymptomatic
8	female, age 53	mastodynia, weight gain, regular menses	169	positive (3.6%)	not performed	nihil	asymptomatic 5 months after diagnosis
9	female, age 47	transient breast dyscomfort	47	borderline (52.4%)[d]	not performed	nihil	asymptomatic 6 months after diagnosis
10	female, age 18	primary amenorrhoea (low body weight, BMI 16 kg/m^2)	117	positive (9.3%)	normal	short course cabergoline	normalisation under cabergoline, rebound after witholding drug; PRL spontaneously normalized 3 years after diagnosis

[a] at time of diagnosis
[b] no adenoma tissue demonstrable histochemically
[c] polycystic ovaries syndrome
[d] macroprolactinaemia confirmed by gel filtration chromatography

Table 2

Prolactin values determined by different assays in the same serum sample, containing 93% macroprolactin (and an actual value for monomeric prolactin of 7.1 µg/l) *(Fahie-Wilson, personal communication).*

Assay	Prolactin value (µg/l)
Bayer/Chiron ACS	26
BioMerieux Vidas	37
Roche Cobas Core	47
Abbott AxSYM	95
Roche Elecsys	143

that are particularly strongly influenced by macroprolactin.

What is the incidence of macroprolactinaemia? On the basis of the – albeit few – epidemiological studies, macroprolactinaemia appears to be rare in the general population. In a Scandinavian study of 660 subjects (healthy probands) Bjoro *et al.* found only one case of macroprolactinaemia; researchers in Japan found similar prevalence data [10, 11].

The true importance of macroprolactinaemia only emerges when one considers exclusively patients whose sera contain abnormally high levels of prolactin. On the basis of a group of 1430 patients with hyperprolactinaemia of several different aetiology (pooled data), it can be estimated that in one out of 5 patients the cause is apparent hyperprolactinaemia (see Table 3). Overall, therefore, it must be assumed that macroprolactin is relatively common in a selected population with hyperprolactinaemia . Both sexes can be affected, although the majority are females; fully asymptomatic patients or subjects with menstrual disorders or galactorrhoea; infertile patients or gravidae; patients with pituitary tumours (including prolactin-secreting adenomas, as pat. 6). The coincidence of macroprolactinaemia and a hormonally inactive pituitary adenoma (or another, possibly artefactual pituitary lesion as it was the case in pat. 1) represents a particularly misleading situation.

What, then, is the clinical relevance of macroprolactin? This calls for discussion of the question of the *bioactivity* of macroprolactin, which has not been adequately settled. While the few available *in*

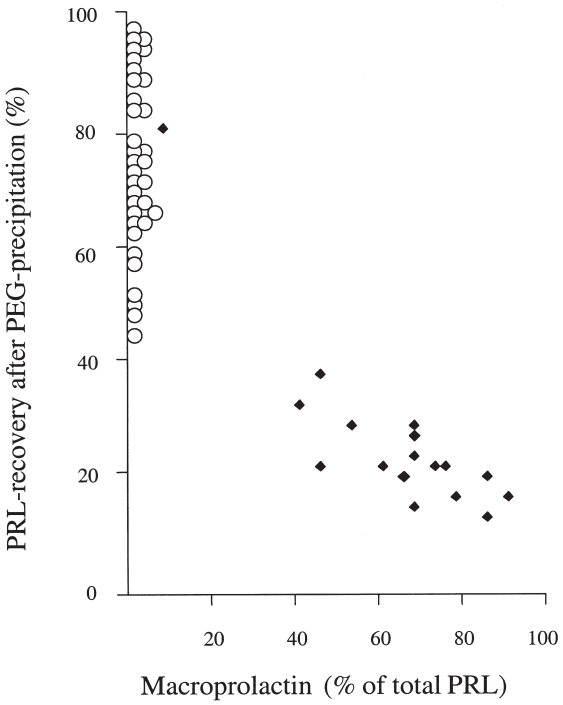

Figure 2

Results of PEG precipitation test in 17 patients with macroprolactinaemia (✦) as a function of the chromatographically quantified macroprolactin content. The control sera without macroprolactin (○) all show a prolactin recovery after PEG of over 40%. The overlap encompasses one single sample containing little amounts of macroprolactin (8% as detected by gas filtration chromatography) *(modified according to [1], by kind permission of the author).*

Table 3

Frequency of macroprolactinaemia in patients with hyperprolactinaemia of variable aetiology.

	n	Proportion of patients with macroprolactinaemia
Hattori, 1992 [5]	20[a]	25%
Hattori, 1994 [8]	208[b]	8%
Hattori, 1996 [13]	105[c]	3%
Fahie-Wilson, 1997 [1]	69	25%
Ahlquist, 1998 [18]	340	16%
Olukoga, 1999 [16]	188	15%
Total	1430	19%

[a] All patients with idiopathic hyperprolactinaemia
[b] 75 of 208 patients had idiopathic hyperprolactinaemia: in 16% of them was macroprolactinaemia detected
[c] unselected gravidae

vitro data are contradictory, macroprolactin appears, in the light of the Nb2 bioassay (rat lymphoma cell cultures, on which prolactin has a mitogenic effect), to exert a receptor-stimulating effect [5, 6]. *In vivo* macroprolactin has only limited activity, if any at all. Many patients with documented macroprolactinaemia have no symptoms related to hyperprolactinaemia. For example, men have been described with normal testosterone values and preserved nocturnal penile tumescence despite highly elevated prolactin values [12]. Further, several women with prolactin values over 200 µg/l achieved conception without therapy [13]. Thus, although some women with macroprolactinaemia can have menstrual disorders or galactorrhoea (e.g. patients 2. and 6. in our series), there are, on the whole, more clinical arguments in favour of absent or at least greatly reduced bioactivity of macroprolactin. An interesting observation suggests that macroprolactin molecules cannot cross the endothelium. In a patient with a prolactin-secreting pituitary adenoma macroprolactin was demonstrated in serum but neither in the CSF nor postop in the pituitary tissue homogenisate, in contrast to monomeric prolactin which was demonstrable in the two last-mentioned localisations (*Ahlquist, personal communication*). Hence macroprolactin may remain in the intravascular compartment and not reach the prolactin receptors at all. Thus macroprolactinaemia is chiefly to be presumed if prolactin is elevated but none of the associated clinical symptoms are present, or if the leading symptoms cannot be satisfactorily explained by hyperprolactinaemia.

A factor of great clinical relevance is neglect of macroprolactinaemia in the differential diagnosis of hyperprolactinaemia; it is probable that a large proportion of so-called *idiopathic hyperprolactinaemias* are in fact caused by macroprolactin. Endocrinology textbooks scarcely mention macroprolactinaemia and survey articles only exceptionally address the subject [14, 15], explaining why even specialists such as gynaecologists and endocrinologists hardly ever include macroprolactinaemia in the work-up of hyperprolactinaemia. The obvious consequences are unnecessary and costly diagnostic procedures such as pituitary imaging, inappropriate treatments, unnecessary follow-up and concern for both patient and clinician. As can be seen from Table 1, disregard of macroprolactinaemia can even result in unnecessary surgery (pat. 1). It should however be mentioned that in the patients of this series most of the work-ups and treatments were carried out at a time before the PEG precipitation test was available.

Why is this clinically important problem still so little known 8 years after it was first described? One reason is that a number of the relevant advances in this field have been published in specialised laboratory journals [1, 10, 17]. Furthermore, hormone measurements are frequently performed as standard groups of multiple analysis (for example, prolactin as part of infertility work-up) and careful correlation of the results with history and clinical data is often omitted. Another reason lies in the diagnostic method. Until recently *no laboratory method* was available for simple diagnosis of macroprolactin. There are very few data about prolactin-suppressive and -stimulatory tests (dopamine infusion, bromocriptin p.o., TRH i.v.) in patients with macroprolactinaemia, and the results are conflicting [8, 13]. If a costly and time-consuming technique such as gel filtration chromatography is needed, the threshold to further work-up of an unclear finding is high. Now, however, the PEG precipitation method offers, in comparison with the gold standard (chromatographic methods), a simple, rapid and cheap method of detecting macroprolactin. It has been estimated that processing of a serum sample by PEG precipitation is 27 times cheaper than gel filtration chromatography [16]. This test is thus highly suitable as a screening method. Using the Wallac Delphia assay, prolactin recovery of <40% after PEG is regarded as a reliable diagnostic criterion for macroprolactin with 100% sensitivity, as

comparisons with gel filtration chromatography have shown; recovery values around 40–50% should, ideally, undergo further chromatographic work-up; values clearly >50% allow macroprolactinaemia to be ruled out [16, 17]. Laboratories however, should validate the PEG technique (using their own prolactin assay) by comparison with gel filtration chromatography.

In summary, macroprolactinaemia is quite frequent in patients with hyperprolactinaemia but is hardly ever considered in work-up or differential diagnosis, a fact which has problematic consequences such as inappropriate treatment, unnecessary diagnostic procedures and aftercare investigations. The specialists involved (particularly endocrinologists, gynaecologists and neurosurgeons) should investigate all patients with so-called idiopathic hyperprolactinaemia for the presence of macroprolactin. Laboratories which offer prolactin determinations should equip themselves for macroprolactin work-up.

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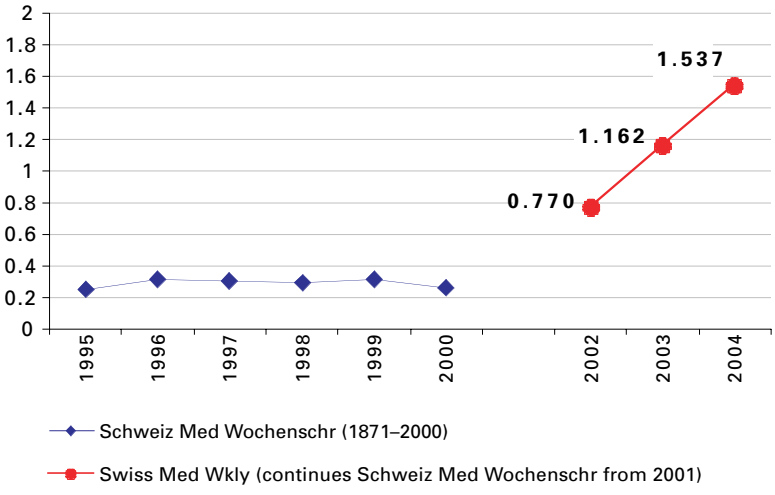

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