**Editorial**

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# Macroprolactin: searching for a needle in a haystack?

DOI 10.1515/cclm-2015-1283

Prolactin (PRL), also known as luteotropic hormone or luteotropin, was originally discovered by Bates and Riddle in the mid 1930s [1]. The human prolactin gene (*PRL*) gene, located on chromosome 6p22.3 [2], encodes for a 220 aminoacids, 23 kDa monomeric protein originating from cleavage of the 26 kDa preprolactin molecule, which is mainly produced by the cells of the anterior pituitary and placenta, precisely by those of the decidualized endometrium [3]. The hormone has essential functions in regulating breast development and milk production in women, but also plays pleiotropic functions as osmoregulation, angiogenesis and immunoregulation [4]. The usual reference range in serum is comprised between 2–18 ng/mL in men and 2–30 ng/mL in women, respectively.

Macroprolactin, also conventionally known as "big-big prolactin", refers to the presence of marked hyperprolactinemia associated with evidence of prolactin-IgG (typically IgG4) circulating complexes displaying a molecular weight of approximately 150 kDa (which is hence 6–7 fold higher that the native molecule) or, less frequently, polymeric aggregate of highly glycosylated prolactin monomers or prolactin-IgA complexes (i.e. non-IgG-type macroprolactin) [5] (Figure 1). The "big-prolactin" is another circulating isoform (usually prolactin dimmers or degradation products of big-big prolactin), with molecular weight comprised between 40 and 60 kDa [6]. In patients with hyperprolactinemia, the serum pattern of prolactin isoforms usually encompasses 60%–90% monomeric prolactin, 15%–30% big-prolactin and 0%–10% big-big prolactin (Figure 1). The condition of macroprolactinaemia is hence defined as predominance (i.e. >30%–60%) of circulating prolactin isoforms with molecular weight >100 kDa [6]. The overall prevalence of macroprolactinemia in the general population ranges between 3% and 4%, but remarkably increases, up to 35%, in patients with hyperprolactinemia [4]. A number of physiological and pathological conditions have been associated with macroprolactinemia, including stress, pregnancy, strenuous physical exercise, pituitary adenomas and cancers, mechanical stimulation of breast, chest wall trauma, hepatorenal disease, primary hypothyroidism, intracranial tumors compressing pituitary stalk or hypothalamus, empty sella syndrome, treatment with prolactin stimulating drugs (i.e. dopaminergic blocking or depleting agents, non-catecholamine dependent agents, H2 receptor blocking agents, tricyclic antidepressants), autoimmune disorders (e.g. thyroid disorders and systemic lupus erythematosus), along with a discrete number (up to one-third of all causes) of idiopathic macroprolactinemias [7]. The condition is frequently asymptomatic, as the binding of endogenous antibodies to epitopes on prolactin molecules which are concomitantly recognized by prolactin receptors ultimately reduces in vivo activity of the hormone, except in patients in whom intermittent dissociation between auto-antibodies and prolactin occurs.

Macroprolactin is usually formed in blood after secretion of monomeric prolactin, and is probably attributable to genetic predisposition or post-translational modifications of the native hormone (e.g. glycosylation, phosphorylation, deamidation), which may led to generation of new epitopes triggering autoantibodies production [7] (Figure 1). Despite macroprolactin retains partial (or total) immunoreactivity against anti-prolactin antibodies used in many commercial immunoassays, it is a biologically inactive form of the hormone. The remarkable increase of circulating prolactin pool in patients with macroprolactinemia is mostly attributable to a discrete number of biological abnormalities, entailing a longer half-life as a consequence of the increased molecular weight (ultimately reducing the renal clearance), the lack of effective binding of big-big prolactin to prolactin receptors (abolishing the physiological hypothalamic negative feed-back mechanism), and the potential competition of prolactin autoantibodies with prolactin molecules for receptor binding [7]. Due to the predominantly benign course of macroprolactinemia, the inherent risk of measuring macroprolactin along with bioactive (free) prolactin may lead to a potential misdiagnosing of hyperprolactinemia, which may then trigger further and unnecessary diagnostic investigations and even inappropriate therapeutic management. As for

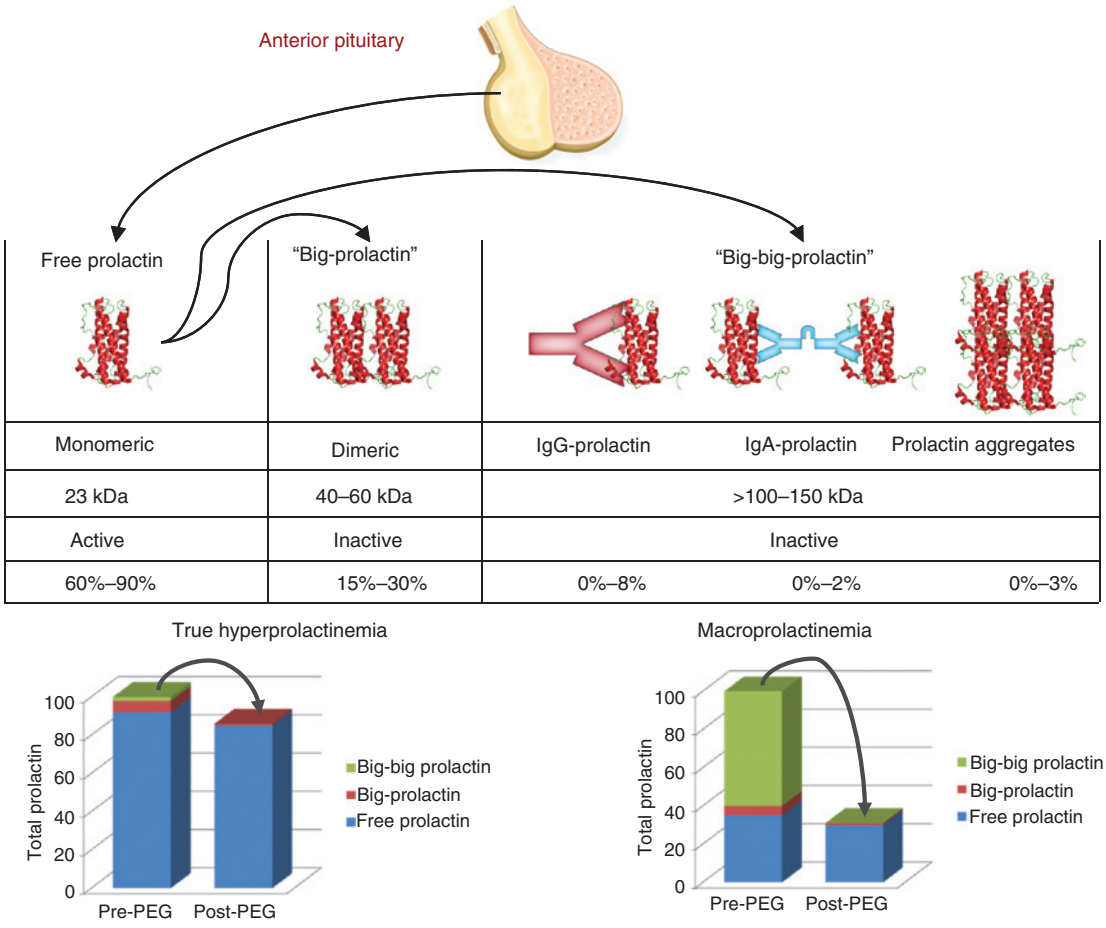


**Figure 1:** Synthesis and structure of free (monomeric) prolactin, big-prolactin and big-big-prolactin.
PEG, poly-ethylene-glycol.

current knowledge, prolactin immunoassays are classified as "low-", "medium-", and "high-interference" methods, depending on number and type of epitopes recognized by the assay antibodies that are already occupied by endogenous anti-prolactin autoantibodies [8]. Very recently, the clinical significance of macroprolactinemia has also been acknowledged by the American Association of Clinical Endocrinologists and the American College of Endocrinology Disease, which concluded that until commercial prolactin assays insensitive to interference by macroprolactin will become available, the possibility of macroprolactin should always be considered when the clinical picture, imaging findings and/or response to treatment are inconsistent [9]. Routine screening for macroprolactin is hence advisable in all patients with hyperprolactinemia, especially in those with lack of symptoms.

At least four different approaches are currently available to detect macroprolactin, each displaying some advantages and limitations. Gel filtration chromatography (GFC) is currently regarded as the gold standard and reference assay, but is mostly labor intensive, time-consuming and expensive. The poly-ethylene-glycol (PEG) precipitation method is a simple, fast and inexpensive approach. Briefly, after mixing serum with 25% PEG, the sample is incubated for a short period of time and then centrifuged for precipitating macroprolactin complexes. The supernatant containing non precipitated prolactin and the untreated serum sample are then both assayed. Although a recovery <40% after PEG precipitation is considered a reliable criteria for diagnosing macroprolactinemia, this technique is plagued by modest specificity, wherein a certain amount of monomeric prolactin may co-precipitate with immunoglobulins, thus yielding a false diagnosis of macroprolactin especially in patients with conditions characterized by increased serum globulin concentrations (i.e. monoclonal gammopathy or

polyclonal hypergammaglobulinaemia) [10]. Alternative, but less widely used, approaches include the use of protein A/G columns, or $^{125}$I-prolacting binding, which are both more labor intensive, expensive and time consuming compared to PEG precipitation.

In this issue of the journal, Hattori et al. publish the results of a large epidemiological investigation, including over 1500 women, who had their serum prolactin values measured with a widely used automated chemiluminescent immunoassay and another manual enzyme immunoassay [11]. The presence of macroplactinemia and IgG-bound prolactin was also extensively investigated by using the reference GFC technique, as well as with Protein G Sepharose and PEG precipitation. The overall prevalence of macroplactinemia was found to be approximately 4% in the study population. Interestingly, the number of women diagnosed with hyperprolactinaemia was lower using the automated chemiluminescent immunoassay compared to the enzyme immunoassay (i.e. 2.4% vs. 3.4%). Non-IgG-type macroprolactin was detected in 20/62 patients with macroprolactinaemia (i.e. 32%), but exhibited a prevalence that was nearly four-fold higher in serum samples in which the presence of macroprolactin was identified by the automated immunoassay than in those in which macroprolactin could not be recognized. In patients with macroprolactinemia, hyperprolactinemia was found to be nearly 10-time lower using the automated chemiluminescent immunoassay compared to the enzyme immunoassay. Last but not least, macroprolactin was confirmed in 42/43 samples with PEG-precipitable prolactin >60% and in 20/45 samples with PEG-precipitable prolactin between 50% and 60%.

Taken together, the results published by Hattori et al. provide a substantial contribution to the challenging issue of identifying macroprolactin in routine laboratory practice, a condition with meaningless clinical significance in patients with normal concentrations of free prolactin. Indeed, the overall prevalence in the general population, confirmed to be as high as 4%, reveals that macroprolactin screening is not like searching for a needle in a haystack. The evidence that PEG precipitation is characterized by a high sensitivity (i.e. up to 100%) but suboptimal specificity for diagnosing macroprolactinemia confirms that all laboratories undergoing macroprolactin screening should establish assay-specific reference intervals obtained from PEG-treated sera. The evidence that the presence of non-IgG-type in patients with macroprolactin may be much higher than previously assumed, up to 30%, is another important aspect which paves the way to redesigning current immunoassays for minimizing the potential cross reaction of free prolactin with macroprolactin (especially that attributable to IgG-prolactin complex), and to the further development of bioassays employing human prolactin receptors for specific measurement of prolactin activity (i.e. the biological response) rather than the serum concentration [5]. Finally, we wish that this paper, along with other contributions, should add fuel to the request to update and improve the current version of the Clinical Practice Guideline on the Diagnosis and Treatment of Hyperprolactinemia, and in particular the section of the guideline dealing with the biochemical diagnosis of hyperprolactinemia when macroprolactin is present [12].

**Author contributions:** All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
**Research funding:** None declared.
**Employment or leadership:** None declared.
**Honorarium:** None declared.
**Competing interests:** The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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