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The high dose hook effect in prolactinomas; following the guidelines?

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Background: Prolactinomas are the most common type of secretory pituitary adenoma. The measurement of prolactin is one of the diagnostic tools in the assessment of patients suspect of a prolactinoma. The Endocrine Society's Clinical Guideline "Diagnosis and Treatment of Hyperprolactinemia" (2011) recommends that in case of discrepancy between a very large pituitary tumor and a mildly elevated prolactin level, dilution of samples need to be performed to eliminate a possible high dose hook (HDH) effect.
Methods: In the current study, implementation of this guideline by clinical pathologists in Dutch clinical laboratories was investigated. In addition, the manufacturers of prolactin assays in the Netherlands were questioned whether they had anticipated in the design of their assays for the possibility of a HDH effect since the report of this effect in 1992.
Results: The majority of the academic hospitals follows the Guideline recommendation. However, unexpectedly in case of regional hospitals, the majority performs dilutions to increase the linear range. From the manufacturer's point of view, besides relying on a large linear range and a low cut-off level above which an (automatic) dilution is performed, four out of five did not introduce additional modifications in the prolactin assays to prevent the HDH effect.
Conclusions: The current study shows that not all laboratories follow the prolactin guideline. The difference in characteristics of the specific prolactin assays as well as sample handling emphasizes the importance of good communication between the clinical pathologist and physician for a correct (laboratory) diagnosis of prolactinoma.

Prolactinomas are the most common type of secretory pituitary adenoma with a prevalence of up to 50% in some series (1). Besides clinical evaluation and imaging studies the measurement of prolactin is one of the diagnostic tools in the assessment of patients suspect of a prolactinoma. Despite several diagnostic options, it can be difficult to differentiate between non-functioning macroadenomas and macropolactinomas (2). This differentiation is of importance because initial therapy of choice for non-functioning macroadenomas is surgery whereas for macroprolactinomas initial therapy are dopamine agonists. In general treatment with dopamine agonists reliably suppresses prolactin levels and induces shrinkage of macroprolactinomas, usually rendering surgery unnecessary. Surgery is reserved for debulking the tumor and alleviating neurological deficit in cases where either the tumor is not responsive to dopamine agonist therapy or if the patient does not tolerate them (1, 3).

In the majority of cases, the biochemical diagnosis of macroprolactinoma is straightforward, because the magnitude of prolactine hypersecretion is usually proportionate to the tumor size. Even though some drugs can induce elevated prolactin levels (e.g., risperidon, metoclopramide), a prolactin level above 5.25 IU/L (250 µg/L) usually indicates the presence of a prolactinoma and a prolactin level greater than 10.5 IU/L (500 µg/L) is considered to be diagnostic of a macroprolactinoma. In some cases of macroprolactinomas prolactin levels are even far above 21.2 IU/L (1000 µg/L). In contrast, marginally elevated prolactin levels (usually below 2.1 IU/L (100 µg/L)) reliably identify these large tumors as nonprolactin-secreting tumors. The marginally elevated prolactin levels are an outcome of the so-called "stalk-effect"; a result of the tumor induced disturbance of pituitary blood flow, thereby limiting the hypothalamic delivery of the prolactin secreting antagonist dopamine.

The Endocrine Society's Clinical Guideline "Diagnosis and Treatment of Hyperprolactinemia" (2011) recommends that in case of discrepancy between a very large pituitary tumor and a mildly elevated prolactin level, serial dilution of serum samples need to be performed to eliminate an artifact that can occur with some immunometric assays leading to a falsely low prolactin value the so called "hook effect" or high dose hook (HDH) effect (3). The guideline also indicates that alternatively to dilution, after prolactin has bound to the first antibody, a washout could be

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performed to eliminate excess unbound prolactin before adding a second antibody (3).
In several hospitals in the Netherlands ordering a (serial) dilution for prolactin as an initial step had become a standard procedure for endocrinologists. The dilution was (often) ordered independent of tumor size and/or knowledge of the initial concentration of prolactin, which does not follow the recommendations of the Endocrine Society's Clinical Guideline. Therefore, we were interested whether and how Dutch laboratories of academic and regional (teaching) hospitals performed prolactin dilutions and how they communicate with their physicians on these results. In addition, the manufacturers of prolactin assays in the Netherlands were questioned whether they had anticipated on the possibility of a HDH effect in their assays, based on the knowledge gained the past few years.

Methods

To obtain information the clinical pathologists of all academic centers in the Netherlands (n=7) responsible for the endocrine diagnostic laboratories were questioned with a standard survey. In addition, clinical pathologists of twelve regional (teaching) hospital laboratories were also questioned with the same survey. The laboratories from the following hospitals were included: Academic Medical Center Amsterdam, Erasmus Medical Center Rotterdam, University of Groningen, University of Maastricht, Radboud University Medical Center, Utrecht Medical Center, VU University Amsterdam; Albert Schweitzer Hospital, Amphia Hospital, Bethesda Hospital, Deventer Hospital, Groene Hart Hospital, Lange Land Hospital, Maasstad Hospital, Medical Spectrum Twente, Queen Beatrix Hospital, Reinier de Graaf Hospital, Sint Elisabeth Ziekenhuis, Sint Franciscus Gasthuis.
The five main manufacturers of prolactin assays in The Netherlands (Immulite 2000 and Advia Centaur, Siemens Healthcare Diagnostics, Tarrytown, USA; AutoDelfia, Perkin Elmer, Turku, Finland; Access/DxI, Beckman Coulter, Brea, USA; Architect, Abbott Wiesbaden, Germany; Cobas Elecsys, Roche Diagnostics GmbH, Mannheim, Germany) were also questioned with a survey about their specific prolactin assay and the possible modifications made in their assay since the first report of HDH effect in 1992.

Results and discussion

The survey amongst the different laboratories showed that all seven academic laboratories and 11 out of 12 (92%) regional laboratories state to perform dilutions of prolactin on either clinical or laboratory (analytical) indication. Half of the regional laboratories indicated to perform dilutions primarily with the purpose to increase linear range instead of identifying HDH effects. All of the academic laboratories indicated to perform dilutions to assess the possibility of a HDH effect.
Concerning the timing of dilution; two regional (17%) and four (57%) of the academic laboratories performed dilution after imaging of the tumor and evidence that there is a discrepancy between a very large pituitary tumor and a mildly elevated prolactin level, as is suggested by The Endocrine Society's Clinical Guideline. Additionally, in two academic laboratories (29%) prolactin dilutions are performed primarily after an observed discrepancy between prolactin levels and imaging but in specific cases the dilution is performed earlier (before imaging of the tumor) based on clinical suspicion of a (macro)prolactinoma. In two regional (17%) and one academic laboratory (14%) prolactin dilutions are always performed when there is clinical suspicion of a prolactinoma but without knowledge on the size of the tumor.
Hence, only 17% of the regional and 57% of the academic laboratories follow The Endocrine Society's Clinical Guideline exactly and perform dilutions only after imaging of the tumor and evidence that there is a discrepancy between a very large pituitary tumor and a mildly elevated prolactin level.
The Guideline also specifies that only specific immunoassays are sensitive to HDH effect, depending on their design. Especially (one-step) two site sandwich immunoassays can be sensitive to excess antigen, since this excess prevents the formation of a "sandwich" complex which is necessary for correct measurement (1, 4). Hence, to overcome a potential HDH effect it is suggested that the assay should be repeated after a 1:100 serum sample dilution (3).
In our current practice at the endocrine diagnostic laboratory of the Rotterdam Academic Center (ErasmusMC) the Siemens Immulite two site immunoassay is used. In our hospital prolactin dilutions are primarily requested by the Endocrine Department, which compromises 6% of their prolactin requests (and 1.5% of the total amount of prolactin requests in the hospital). Analyses showed that for all prolactin dilutions performed in the last 10 years (2003-2013) no sample showed a high dose hook effect, even at prolactin levels up to 1200 IU/L (57 µg/L), which is far above the limit above which samples are diluted automatically (3.15 IU/L, table 1) and the claim of no HDH effect of the manufacturer (435 IU/L, table 1).
Based on the historical knowledge of the HDH effect for prolactin (1, 2, 5-11) and the recommendations of The Endocrine Society's Clinical Guideline (3), different methods for elimination of a HDH are acknowledged:

- introduction of an extra washing step to remove excess antigen before adding the signal antibody (two step technique) (4, 12)
- adding more labelled antibodies (this does not always eliminate the HDH effect) (13)
- computerized kinetic rate analysis in order to establish the need to dilute (14)
- dilution of the sample

The specifications of different prolactin assays distributed in the Netherlands were analyzed for the possible methods for prevention of a HDH effect. The survey included in total five manufacturers (table 1), all of which were aware of the HDH effect.
Four out of five are currently providing a one-step two site assay, without a washing step to remove prolactin excess. One manufacturer provides a two-step two

Table 1. Information of 6 prolactin assays and their characteristics (obtained from manufacturer inserts 2015).

Manufacturer	Apparatus	Method	HDH possible?	Specific prevention?
Siemens	Immulite	Two site sandwich immunoassay	Yes	Automatic dilution > 3.15 U/L No HDH up to 435 U/L
Siemens	Centaur	Two site sandwich immunoassay	Yes	Automatic dilution > 4.24 U/L No HDH up to 636 U/L
Perkin Elmer	AutoDelfia	Two site sandwich immunoassay	Yes	No HDH effect up to 90 U/L
Beckman	Access/Dxl	Two site sandwich immunoassay	Yes	Automatic dilution > 4.24 U/L No HDH effect up to 636 U/L
Roche	COBAS	Two site sandwich immunoassay	Yes	Automatic dilution > 10 U/L No HDH up to 270 U/L
Abbott	Architect	Two site sandwich immunoassay, with specific washing step	No	Automatic dilution > 4.24 U/L

site assay with an additional washing step after incubation of the sample with the first antibody, which theoretically circumvents the HDH effect.
See Table 1 for information about the five specific assays used in the Netherlands and their cut off points.

Conclusion

The presence of a high dose hook effect for prolactin in two automated immunoassays was first described in 1992 (4). Comtois et al (15) reported the first patient in whom the hook effect lead to misdiagnosis of a large macroprolactinoma. The following years a series of cases was published on the high dose hook effect in patients with macroprolactinomas (1, 2, 5-11), in which dilution of samples was most often advocated as the solution to this effect.
As previously, described (3) a dilution for prolactin is advocated when there is a discrepancy between imaging studies of a large pituitary adenoma and relatively low prolactin levels. In the current study it was shown that there is a difference in handling possible HDH samples amongst 7 academic and 12 regional laboratories in the Netherlands. In the majority of the academic laboratories samples are diluted when there is a discrepancy between a very large pituitary tumor and a mildly elevated prolactin level; whereas half of the regional laboratories indicated that samples are diluted with the purpose to increase the linear range. However the specifications of the current prolactin assays show that these assays have a large linear range due to automatic dilutions above the cut-off levels. Most manufacturers have set this cut-off point at a relatively low level thereby minimizing the need to manually dilute the samples. Hence, based on these specifications, laboratories need to focus on samples with a prolactin concentration below the cut-off concentration in combination with a large pituitary tumor; these samples need to be (manually) diluted. Besides a large linear range and a low cut-off level for an extra dilution step, four out of five manufacturers did not change the one-step design assay since the first report of the HDH effect in 1992. They claim that the possibility of a HDH effect is minimized by the design of their assay, e.g. a large linear range of the assay and a low cut off concentration above which a dilution is automatically or manually (depending on the assay) performed. Although a large linear range minimizes the possibility of a HDH effect, it does not completely circumvent it. At extremely high concentrations of prolactin, e.g. in very large macroprolactinomas, the HDH effect can still occur. Hence, most manufacturers did not actively change the assay design for the detection of a HDH effect; thereby leaving the responsibility at the level of the clinical pathologist, who should in addition inform its physicians about these effects. Thus, although the Endocrine Society's Clinical Guideline states otherwise ("Newer assays may obviate this problem (High Dose Hook effect)"); this is not the case for the assays distributed in the Netherlands. Our advice is to perform dilutions below the cut off concentrations of the used assay when imaging is showing a large pituitary tumor. When the patient concentration is above the cut off concentration no dilutions are necessary because in most assays samples are diluted automatically in that concentration range. If this automatic dilution is not possible, a manual dilution is necessary.
The possibility of a HDH effect depends on the design of the assay, therefore knowledge of the assay used is important. Despite the reports of the occurrence of the HDH effect, in our large referral hospital, a HDH effect for prolactin because of a large macroprolactinoma was not observed in the last ten years. Therefore, although there is a possibility of misdiagnosing a large prolactin-secreting macroadenoma due to a HDH effect, an initial dilution (without imaging studies)

should not be performed. In addition to a reduction in (manual) sample handling for dilutions when following the guidelines, this also results in a more cost-effective approach since there will be a reduction in the number of prolactin measurements per patient.
Since most of the prolactin assays used in the Netherlands still have a one-step approach, knowledge of the specific assay used in the hospital is necessary. Most of the physicians are not aware of the characteristics of the specific prolactin assays. Therefore, a good communication between physicians and clinical pathologists is necessary.

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References

1. Fleseriu M, Lee M, Pineyro MM, Skugor M, Reddy SK, Siraj ES, Hamrahian AH. Giant invasive pituitary prolactinoma with falsely low serum prolactin: the significance of 'hook effect'. J Neuro-Oncol. 2006; 79: 41-43.
2. StJean E, Blain F, Comtois R. High prolactin levels may be missed by immunoradiometric assay in patients with macroprolactinomas. Clin Endocrinol. 1996; 44: 305-309.
3. Melmed S, Casanueva FF, Hoffman AR, Kleinberg DL, Montori VM, Schlechte JA, Wass JA. Diagnosis and treatment of hyperprolactinemia: an endocrine society clinical practice guideline. J Clin Endocr Metab. 2011; 96: 273-288.
4. Haller BL, Fuller KA, Brown WS, Koenig JW, Eveland BJ, Scott MG. Two automated prolactin immunoassays evaluated with demonstration of a high-dose hook effect in one. Clin Chem. 1992; 38: 437-438.
5. Barkan AL, Chandler WF. Giant pituitary prolactinoma with falsely low serum prolactin: the pitfall of the "high-dose hook effect": Case report. Neurosurgery. 1998; 42: 913-915.
6. Schöfl C, Schöfl-Siegert B, Karstens JH, Bremer M, Lenarz T, Cuarezma JS, Samii M, et al. Falsely low serum prolactin in two cases of invasive macroprolactinoma. Pituitary. 2002; 5: 261-265.
7. Delgrange E, de Hertogh R, Vankrieken L, Maiter D. Potential hook effect in prolactin assay in patients with giant prolactinoma. Clin Endocrinol. 1996; 45: 506-507.
8. Petakov MS, Damjanovic SS, Nikolic-Durovic MM, Dragojlović ZL, Obradović S, Gligorović MS, Simić MZ, et al. Pituitary adenomas secreting large amounts of prolactin may give false low values in immunoradiometric assays. The hook effect. J Endocrinol Invest. 1998; 21: 184-188.
9. Frieze TW, Mong DP, Koops MK. "Hook effect" in prolactinomas: case report and review of literature. Endocr Pract. 2002; 8: 296-303.
10. Unnikrishnan AG, Rajaratnam S, Seshadri MS, Kanagasapabathy AS, Stephen DC. The 'hook effect' on serum prolactin estimation in a patient with macroprolactinoma. Neurol India. 2001; 49: 78-80.
11. Yener S, Comlekci A, Arda N, Men S, Yesil S. Misdiagnosis due to the hook effect in prolactin assay. Med Prin Pract. 2008; 17: 429-431.
12. Dahlmann N, Brensing KA, Klingmuller D, Bidlingmaier F. Hook-effect in a patient with a gonadotropin-secreting tumor. Clin Chem. 1990; 36: 168.
13. Khosravi MJ. Shifting the hook effect in one-step immunometric assays. Clin Chem. 1990; 36: 169-170.
14. Hoffman KL, Parsons GH, Allerdt LJ, Brooks JM, Miles LE. Elimination of hook-effect in 2-site immunoradiometric assays by kinetic rate analysis. Clin Chem. 1984; 30: 1499-1501.
15. Comtois R, Robert F, Hardy J. Immunoradiometric assays may miss high prolactin levels. Ann Intern Med. 1993; 119: 173.

Samenvatting

Boesten LSM, Krabbe JG, de Rijke YB. High-dose-hook effect in Prolactinomas: wie volgt de richtlijnen? Ned Tijdschr klin Chem Labgeneesk. 2015; 40: 230-233.
Prolactine-secreterende adenomen vertegenwoordigen 40% van de hypofysaire tumoren. De Clinical Guideline “Diagnosis & Treatment of Hyperprolactinemia” van de Endocrine Society, beveelt een verdunningsreeks aan bij een discrepantie tussen een grote tumor en een mild verhoogd prolactine ter voorkoming van een mogelijk High-Dose-Hook (HDH) effect. In deze studie wordt voor de Nederlandse situatie onderzocht hoe Laboratoriumspecialisten Klinische Chemie (LKC) deze richtlijn geïmplementeerd hebben. Daarnaast zijn de prolactine meetmethoden op de Nederlandse markt onderzocht.
De meerderheid van de academische ziekenhuizen volgt de richtlijn aanbevelingen; in tegenstelling echter tot de perifere ziekenhuizen. Daarnaast heeft het merendeel van de fabrikanten de prolactine meetmethoden niet gewijzigd sinds de eerste beschrijving van het HDH effect.
De huidige studie laat zien dat niet alle laboratoria de richtlijn volgen. De verschillende prolactine meetmethoden en de wijze waarop de LKC deze diagnostiek hebben ingericht, benadrukken het belang van goede communicatie tussen de LKC en de behandelend arts voor een juiste diagnostiek van een (macro) prolactinoom.