Clinical case

The hook effect in calcitonin immunoradiometric assay: A case report

L’effet crochet dans le dosage radio-immunologique de la calcitonine, à propos d’un cas

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Abstract

The hook effect, which has long been detected and documented for immunoradiometric assays (IRMA) such as those measuring prolactin or thyroglobulin, occurs when the serum antigen level is extremely high, thus inducing a bias in the methodology of measurement. Results. – We report the case of an 80-year-old man with confirmed medullary thyroid carcinoma (MTC). In the case reported here, the clinical status of the patient contrasts with his tumor antigen, serum calcitonin (CT), concentrations. The measured increased CT concentrations revealed the presence of a hook effect. This phenomenon occurs due to an excess of antigen during the one-step IRMA where the signal antibodies, bound to the non-captured antigens, are washed out during the measurement, inducing the loss of signal. Aiming to prevent the “hook effect”, successive dilutions of the same sample of serum were done. Conclusions. – Previous studies have shown when one-step IRMA reveals high concentrations of a tumor serum antigen (i.e. prolactin or thyroglobulin), a two-step IRMA or a systematic 1:10 dilution of the serum sample prevents the formation of the “hook effect”. In our case report, the CT “hook effect” formation was prevented by performing serial dilutions of the serum sample.
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Résumé

L’effet crochet, décrit depuis longtemps pour des dosages radio-immunologiques comme ceux de la prolactine ou de la thyroglobuline, apparaît lorsqu’un taux d’antigènes très élevé induit un biais dans la méthode de mesure. Cas clinique. – Dans notre étude, l’état clinique du patient ne correspondait pas au taux mesuré de thyrocalcitonine sérique. Nous avons donc réalisé des dilutions successives d’un même échantillon de sérum, qui ont montré des valeurs de concentration croissantes, signant un effet crochet dû à un excès d’antigènes pendant ce dosage radio-immunologique en une étape. De fait, les « anticorps-signal » liés à des antigènes non capturés peuvent être éliminés au cours du dosage, induisant une perte de signal. Ainsi, pour éviter l’effet crochet ont été adoptées, telles que les dosages radio-immunologiques en deux étapes ou la dilution systématique au 1:10 des échantillons suivie d’une mesure sur l’échantillon dilué et non dilué. Dans le cas de la thyrocalcitonine, dont les augmentations de taux de thyrocalcitonine sérique sont généralement pas extrêmes, et pour laquelle peu d’effets crochets ont été jusqu’à ici décrits, nous suggérons la dilution au 1:10 des échantillons, soit systématiquement, soit en fonction du statut clinique.
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1. Hook effect in calcitonin immunoradiometric assay: a case report

Calcitonin (CT), or thyrocalcitonin, is a peptide hormone synthesized and secreted by the para-follicular C-cells of the thyroid gland, under serum calcium control. It is a reliable and sensitive tumor marker for medullary thyroid carcinoma (MTC), a rare malignant tumor, accounting for 3 to 5% of all thyroid cancers.

A high concentration of serum CT (> 100 ng/L) before surgery confirms the diagnosis and, after the treatment (a total thyroidectomy with cleansing of the lymph nodes), the level of CT has to decrease back to the normal range.

Therefore, the measurement of CT is not only used for the diagnosis of MTC, but also in the postoperative follow-up of
patients with MTC. It is also widely used as an index of the response of MTC to systemic treatment, although the results do not always parallel the evolution of tumor burden.

Furthermore, recent protocols using CT doubling time (CDT) and serum CT concentrations in prognostic assessment of MTC reveal the need for the most reliable measurement method or IRM of serum CT.

2. Clinical case

We report the case of an 80-year-old man, known by clinicians as a MTC carrier despite lack of previous measurement in our laboratory. In February 2010, his first measurement of serum CT level in our laboratory was 306 ng/L (with a one-step IRMA method [BIOSOURCE CT-U.S.-IRMA kit]). The calibration curve was linear between 7.4 and 450 ng/L.

The results, which appeared to be falsely low, were not consistent with the patient’s clinical state. After a MTC diagnosed in 2002, and a thyroidectomy with cleansing of the lymph nodes, his serum CT level never returned to a normalized value: from 800 ng/L after the surgery in 2002, it progressively increased to 31,000 ng/L in July 2009. Performing a second surgery was refused by the patient. Today, he has residual lymph node disease with vertebral column metastasis.

Results of the February control dosage, with serial dilutions, are given in Table 1, lines 1 and 2.

The control dosage in the undiluted (1:1) sample was different (>450 ng/L) from those of the initial dosage (306 ng/L), showing a dose variability of serum CT. The serum CT level in the undiluted sample was lower than that of the 1:10 and 1:100 dilutions. Beyond the 1:100 dilution, all results were almost similar between 36,000 ng/L and 43,000 ng/L. The above measurements revealed the presence of a hook effect.

Which value was to be considered reliable? The serum concentration that remained unchanged (39,860 ng/L) throughout at least two successive dilutions was considered to be reliable and was found in the middle of the calibration curve. The serum CT level of 39,800 ng/L, measured at 1:200 dilution, was reported to the clinician.

Three months later, in May 2010, the next measurement of the same patient CT level was directly performed through serial dilutions. The results are given in Table 1, lines 3 and 4.

Once again the value obtained for the 1:200 dilution, 29,200 ng/L, was chosen to be reported. Further serum CT level recordings of the same sample were done at 1:100, 1:200 and 1:500 dilutions. These recordings remained in the area inside of the calibration curve.

3. Discussion

The hook effect is a well-known side effect of immune analysis encountered at diagnosis and monitoring of endocrine diseases. Prolactin [1–4] and thyroglobulin [5] titrations have specially suffered from “hook effect” interference.

This effect happens when a very high level of antigen exists in a serum leading to a falsely low analyte titration. This low marker concentration is not in line with the evolution of tumor burden.

Hook effect interference occurs during a one-step immunoradiometric assays (IRMAs). The baseline of this type of IRMA is that of the antigen. It is targeted by two monoclonal specific antibodies. One of them is a capture antibody, attached to the plastic of the reaction vial, and the second one is a signal antibody, linked to a radiomarker (I125). Both of them, during the incubation period with the serum, bind the antigen in a “sandwich” way. After a wash step, “sandwich complexes” remain inside of the vial and the radiomarker of the signal antibody is used to measure the radioactivity, directly proportional to the concentration of the analyte.

However, when the concentration of antigen is extremely high (beyond the last point of the calibration curve), the binding capacity of the two antibodies is affected and becomes saturated. As a result, most of the analyte is lost during the wash step. This leads to the loss of signal antibody, which is linked to the analyte and not to the testing vial. Thus an erroneously low level of antigen is detected. (Fig. 1: a simplified view of the hook effect in the IRMA one-step assay [6]).

Suspicion of a hook effect indicates the need for additional measurement after serial dilution of the patient’s serum sample.

Moreover, the measured concentration can be even more misleading as it tends to become part of the calibration curve. In fact, beyond a high level of antigen, the signal drops back, and can fall into the calibration curve (Fig. 2, Zone B, [6]). This confusing situation doesn’t allow the detection of an interference error. On the other hand, when the concentration is falsely low but outside of the calibration curve (Fig. 2 Upper than zones A and B), there is a suspicion of a hook effect. This indicates the need for additional measurement after serial dilution of the patient’s serum sample.

There are several ways of preventing the hook effect mistake.
The first one consists of a two-steps immuno-radiometric method. The first step links the analyte to the capture antibody, which is coated on a vial. The analyte in excess is then washed up before the second step when the signal antibody is incubated with the analyte-capture antibody complex, forming “sandwiches”. After a final wash, the “analyte-antibodies” complexes remain, and the signal is measured. This method prevents the hook effect by discarding the antigens in excess, avoiding the “waste” of radiosignal due to the analyte binding to the signal antibody without being linked to the capture antibody. However, this method does not lead to a “real” concentration. The signal measured at the end of the analysis is the concentration of capture antibodies coated on the tube and not the one bound to the analyte. The analyte concentration in the sample is then “superior to” the signal measured.

Another possibility is to use a systematic 1:10 dilution of the sample, titrated in parallel with the pure sample, as it is realized for the PRL titration. A concentration measured within the diluted sample higher than the undiluted one proves the existence of a hook effect. In this case, several other dilutions will be performed to obtain a more accurate value. Unfortunately, this is not always routinely feasible, for it requires more reagents and time, thus being more expensive.

A possible alternative could be the use of a dilution on an individual basis. For known patients who never showed any hook effect, on postoperative follow up, a measurement can be realized on pure serum. If CT concentration is undetectable, the result has to be trusted. If CT concentration remains detectable, a measurement after dilution has to be performed to exclude a hook effect. Nevertheless, for patients unknown to the laboratory, or known to present a hook effect, a systematic 1:10 dilution has to be realized for every CT concentration assay.

This approach has been developed for some antigens such as thyroglobulin or prolactin. The pathologies explored by these tumor markers, prolactin macroadenomas and thyroid cancers, can lead to extremely high levels of antigen. The hook effect has been long documented for these pathologies.

The hook effect has not often been suspected, detected and described in previously published MTC studies [7–9]. This is mainly because MTC is rare and the observed CT concentrations are often lower than 10 pg/ml. On the contrary, the reported high concentrations of other serum antigens such as prolactin and thyroglobulin have lead to the detection and prevention of the hook effect.

Considering the diagnostic value of serum CT concentration (surgery being mandatory when the titration has reached twice the first concentration), and its prognostic value in the postoperative follow-up of MTC, it seems necessary to establish a reliable method for measuring an accurate concentration.

The third approach being done on an individual basis seems to yield more satisfactory and reliable results. It is routinely feasible in the laboratory, and is not expensive.

4. Conclusion

If the hook effect is well known for antigens such as thyroglobulin and prolactin, we here expose a hook effect observed in a dosage of serum CT. This pitfall can be encountered for any antigen measured with one-step IRMA.

Biologists and clinicians should be aware of this phenomenon. Our study suggests that the hook effect can alter postoperative prognosis in MTC patients. The measurement of serum CT level as an index of the response of MTC to systemic treatment may not always be in line with the evolution of tumor burden. Our results are consistent with those reported by Le Boeuf et al. [7]. In order to prevent the hook effect, we suggest a systematic approach: a serum CT measurement either through a 1:10 dilution of the sample or through a 2-step IRMA for all patients or for targeted ones (unknown or already documented with very high serum levels of CT). This measurement becomes a reliable prognostic guide to the short-term and long-term management of MTC patients while the patient’s clinical state has been taken into account. Therefore, we stress on the need for a dialog between the CT testing laboratory team and the clinician.

Disclosure of interest

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**References**


