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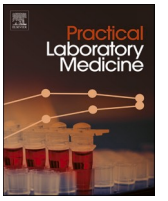

Double trouble: Unmasking two hook effects on Siemens Atellica® - Total PSA and total hCG assays

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ABSTRACT

The "hook effect" or "prozone phenomenon" occurs when the concentration of a particular analyte saturates the antibodies used in the test, resulting in falsely low or negative results despite the presence of high analyte concentrations. We report two recent cases of hook effect encountered with a widely used immunoassay analyzer, the Siemens Atellica® IM1600. The first case involves a patient with advanced metastatic prostate cancer whose total PSA (tPSA) concentration dropped dramatically from his last biological control. The second case concerns a pregnant woman whose total HCG (ThCG) levels were also subject to the hook effect and who was found to have a molar pregnancy. In both cases, a dilution step enabled to overcome this analytical concern and to obtain a correct result. In addition, a comparison of the sensitivity of different immunoassay analyzers to this phenomenon was carried out. To avoid this analytical error, an additional dilution step should automatically be performed when there is a clinical suspicion of elevated levels of tumor or hormone markers. Finally, the most affected manufacturers should adapt their assays, accordingly.

1. Introduction

The "hook effect" or the "prozone phenomenon" in immunoassays is a phenomenon that occurs when the concentration of a particular analyte (such as a hormone or protein) is so high that it saturates the antibodies used in the assay. This can lead to a falsely low or negative result, despite the analyte is present at high concentrations. The hook effect is typically observed in sandwich immunoassays, and can be corrected by diluting the sample [1]. In this article, we report two recent cases of hook effect encountered with a widely used immunoassay analyzer.

2. Material and methods

Samples were measured with the Siemens Atellica® IM1600 analyzer, (Siemens Healthineers, Erlangen, Germany). The first one concerns the Atellica® IM total PSA assay and the second one, the Atellica® IM Total hCG (ThCG) assay. For these two samples, a dilution was performed to highlight the presence of a hook effect.

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To know if other manufacturers were also impacted by this analytical pitfall, samples were frozen and were sent for comparison of the PSA measurement using three different analyzers; the Cobas® e602 (Roche Diagnostics, Mannheim, Germany), the Alinity®I (Abbott Laboratories, IL, USA) and the VITROS® 4600 (Ortho Clinical Diagnostics, New Jersey, U.S.) (Table 1).

The Cobas® e602 and Vitros® 4600 analyzers use the same test principle as the Atellica®; a one-step sandwich immunoassay, while the Alinity® uses a two-step sandwich immunoassay with an additional washing step before adding the second antibody, which removes excess antigens.

3. Results

3.1. Case 1

PSA measurement was requested for a 78-year-old man with advanced castration-resistant prostate cancer with bone and lymph node metastases, as part of the follow-up of his disease. Patient had undergone several chemotherapy and hormonotherapy treatments (Abiraterone acetate, Docetaxel, Degarelix, Cabazitaxel) but had progressive disease for many years. Surprisingly, at the last patient's follow-up, his PSA value dropped drastically compared to its last biological control, performed 1 month before (from 4853.94 ng/mL to 78.0 ng/mL). However, following interdisciplinary discussion, this result was not consistent with his clinical condition. Therefore, we decided to perform a dilution and the PSA result was 9520 ng/mL, after correction for the dilution factor **(Table 1)**.

After sending the sample to other laboratories for comparison, it was found that all other analyzers showed a PSA concentration >100 ng/mL on the first measurement, which would commonly require a dilution step to obtain the precise value, therefore probably leaving a potential hook effect.

According to the kit inserts of the different manufacturers (Table 1), the Atellica® IM seems more prone to the hook effect because the amount of detection antibodies used in the reaction process is lower. Moreover, the threshold value up to which the manufacturer did not observed a hook effect is also lower than the others.

3.2. Case 2

The second case involved a 30-year-old woman, 13 weeks pregnant, who was admitted to the emergency department for hyperemesis gravidarum. On admission, an hCG assay was performed on the Atellica® IM1600 and a concentration >200,000 U/L was observed.

One week later, a second hCG measurement was performed for the follow-up. The concentration obtained was this time very low (992 U/L) compared to the previous value obtained. No automatic dilution was performed, regarding the limit of linearity is 1000 U/L. This result was not consistent with the clinical and the ultrasound test which showed a good fetal viability. Therefore, one week later, a third hCG test was performed and once again, the concentration obtained was very low (866 U/L).

After serial dilutions of this last sample, hCG levels rose to over 10,000,000 U/L. We therefore concluded that the last two hCG determinations were subject to hook effect.

After several additional examinations, trisomy 21 was detected at amniocentesis and a molar pregnancy was suspected. The patient had to undergo a medical termination of pregnancy (MTP) and a follow-up of the decrease of the hCG level was carried out until normalization. Normalization of hCG level (<2.0 U/L) occurred 105 days following MTP.

All hCG measurements were performed with the "Atellica IM ThCG" kit. The assay method involves a sandwich immunoassay in one-step and according to the insert kit, this test can be prone to the hook effect for hCG concentration >400,000 IU/L.

Unfortunately, we did not have enough volume of this sample to check if the other analyzers were also impacted by this analytical issue.

4. Discussion

When hook effect happens, the excess of antigens may saturate both captured and signal antibodies, leading to the hindrance of sandwich formation, providing therefore a falsely low result. The higher the concentration, the more saturated the antibody binding sites will be [2]. This effect occurs mainly in one step immunoassays where antigen, antibody and the sample are all incubated

Table 1
Comparison of PSA assay on 4 analyzers.

Analyzer	Atellica® IM1600 (Siemens Healthineers)	Cobas®e602 (Roche Diagnostics)	Alinity®I (Abbott Laboratories)	Vitros® 4600 (Ortho Clinical Diagnostics)
Kit	tPSA Atellica IM	tPSA Elecsys	tPSA Alinity i	tPSA
Measuring range	0.01–100.00 ng/mL	0.003–100.00 ng/mL	0.025–100.00 ng/mL	0.01–100 ng/mL
Absence of Hook effect until: (as noticed in the insert kit)	2500 ng/mL	17,000 ng/mL	48,000 ng/mL	11,048 ng/mL
Test principle	Sandwich immunoassay in one step	Sandwich immunoassay in one step	Sandwich immunoassay in two-step	Sandwich immunoassay in one step
Native sample	78.0 ng/mL	>100 ng/mL	>100 ng/mL	>100 ng/mL
After dilution (dilution factor)	9520 (100x)	9168 (100x)	8231 (100x)	7970 (100x)

simultaneously. Therefore, its incidence can be reduced by adding a washing step.

In some pathologic situations, such as tumors that secrete much higher levels of peptide markers than normally found, hook effects are more common. In the case of prostate-specific antigen (PSA), the hook effect is more often seen in advanced prostatic adenocarcinoma with bone metastases due to possible extremely high serum PSA levels [3]. Kittanakom et al. reported a similar case in 2018 on the Siemens Centaur platform, which use the same reagents as the Siemens Atellica® IM analyzer [4].

In the same way, hydatidiform mole can produce abnormally high amounts of hCG leading to a potential hook effect. Indeed, several cases of hCG hook effects have been described, most often related to molar pregnancies [5–7].

Recently, a similar case of hCG hook effect on the Atellica IM ThCG kit has been reported in a patient who also had hyperemesis gravidarum with molar pregnancy [6]. This case highlighted the increased susceptibility of the ThCG Atellica IM kit to the hook effect, which was not observed with two alternative analyzers (Roche Cobas e801® and Abbot Alinity I®). Similarly, they observed a value (946 U/L) lower than the automatic dilution cutoff.

A possible improvement of these kits (tPSA Atellica IM and ThCG Atellica IM) would be a revision of the analytical protocol, like using higher concentration of antibodies or a higher sample pre-dilution factor, to avoid the saturation of the capture antibodies.

5. Conclusion

In conclusion, an additional dilution step should automatically be performed when there is a clinical suspicion of elevated levels of tumor or hormonal markers. The communication between clinicians and clinical biologists is key to prevent such analytical error. Some manufacturers should take more into account the risk of hook effect for tumor markers and adapt their assay steps or formulation, accordingly.

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CRediT authorship contribution statement

Meryem Benamour: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. **Pauline Brouwers:** Data curation, Formal analysis. **Arnaud Nevraumont:** Formal analysis. **Tatiana Roy:** Formal analysis, Project administration. **Jean-Louis Bayart:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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