Letter to the Editor

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Falsely low beta-hCG results in pregnant woman on Siemens Atellica: don't forget the "hook effect"

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To the Editor,

Human chorionic gonadotropin (hCG) is a well-known marker for pregnancy or gestational trophoblastic diseases, daily assayed in most of the laboratory medicine [1]. However, the serum hCG concentration may be sometimes misreported. Indeed, falsely low results can occur and may directly influence patient care management.

A 27-year-old pregnant woman (gestational age: 11 weeks and two days) was admitted to the emergency department for uncontrollable vomiting unresponsive to antiemetic for three weeks, and a five-kilogram weight loss.

At admission, the physical examination showed an absence of metrorrhagia and no abdominal pain. Laboratory tests indicated a mild inflammatory syndrome (C-reactive protein: 13.6 mg/L) associated with hepatic cytolysis (aspartate transaminase: 76 U/L, alanine transaminase: 150 U/L) and hyperthyroidism (thyroid stimulating hormone: undetectable, free thyroxine: 63.9 pmol/L and free triiodothyronine: 25.3 pmol/L). The hCG assay was positive, but low for gestational age (948 IU/L). After these results, the patient was hospitalized in the gynaecology unit for hyperemesis gravidarum with a suspected miscarriage or molar pregnancy. Then, an ultrasound scan was performed, confirming a uterus suggestive of molar pregnancy. A control hCG assay was realized two days after hospitalization and showed a non-changing result (hCG: 976 IU/L). Nevertheless, it's interesting to note that these two results were situated just below the limit of measure (i.e. 1,000 IU/L), which automatically induce a dilution by the analyser.

Following the discrepancy between the clinical presentation suggestive of molar pregnancy and the hCG levels, which remained low and stable, dilutions were carried out on the patient's sera by the laboratory, to highlight a potential analytical interference. The hCG assays were performed using the Siemens hCG test Atellica IM Total hCG (ThCG) (Siemens Healthineers, Erlangen, Germany) on Atellica IM1600 analyser (Siemens Healthineers, Erlangen, Germany).

Analysis of diluted samples (dilutions performed to 1:1,600), taken on admission and after two days of hospitalization, provided the hCG concentrations of 1,437,304 IU/L and 1,375,411 IU/L respectively. These concentrations were approximately 1,500 times higher than initially reported, and a "hook effect" was therefore suspected to explain these discrepancies.

In order to verify the presence of this interference on different analysers, these two sera were also analysed with two additional methods. Unlike our routine method on Atellica IM1600 analyser, "hook effect" did not affect the results on these two analysers. Indeed, results obtained on Cobas e801 (Roche Diagnostics, Mannheim, Germany) and Alinity I (Abbott Laboratories, IL, USA) analysers were above the measuring interval of the respective assay, requiring dilutions to obtain a quantitative hCG value.

Comparison of characteristics of the three hCG methods (Table 1) was made to understand these analytical divergences. Differences were marked in the measuring intervals of the assays. Indeed, measuring interval on Atellica (1,000 IU/mL) was ten and fifteen times smaller than Roche (10,000 IU/L) and Abbott's (15,000 IU/L) tests respectively. All three kits used a sandwich assay, but Abbott Alinity's method was performed in two-step using two consecutive washes while Siemens and Roche employed a one-step technique. Moreover, hook effect was noticed by manufacturers as above 400,000 IU/L in Siemens's insert kit and above 750,000 IU/L in Roche's insert kit, whereas no hook effect was claimed by Abbott. The hook effect on hCG assay has been previously reported in the scientific literature on several analysers [2]. However, no studies

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Table 1: Comparison of characteristics of the three hCG methods.

Analyser	Atellica IM1600	Cobas e801	Alinity I
Manufacturer	Siemens healthineers	Roche diagnostics	Abbott laboratories
Assay name	Atellica IM total hCG (ThCG)	Elecsys HCG+β	Alinity i total β-hCG reagent kit
Measurement interval (IU/L)	2.0–1,000	0.2–10,000	2.30–15,000
Hook effect noticed in insert kit	>400,000 IU/L	No observed until 750,000 IU/L	Unspecified
Standardization	WHO international standard 4th IS chorionic gonadotropin, human NIBSC code: 75/589	WHO international standard 4th IS chorionic gonadotropin, human NIBSC code: 75/589	Unspecified
Assay method	Sandwich in one-step	Sandwich in one-step	Sandwich in two-step
Sample 1 undiluted hCG results, IU/L	946	>10,000	>15,000
Sample 2 undiluted hCG results, IU/L	976	>10,000	>15,000

published on the subject have reported any high-dose effect on the Atellica IM1600 analyser.

To complete the investigations and try to understand the range of this interference on Atellica IM1600, twenty-one sera with hCG concentrations between 3 IU/L and 1,400,000 IU/L were analysed using our routine hCG method for an assessment of the relative light unit (RLU) in regard of hCG concentration in serum (Figure 1). As claimed by Siemens in the hCG test insert kit, a decrease of RLU was observed for hCG concentration above 400,000 IU/L. This confirming the suspected hook effect obtained for the sera assayed at admission and two days after hospitalization of the patient.

In this case, the patient's hCG was, in reality, approximately 1,500 times higher than the limit of linearity (LoL) of the method. Normally, the Atellica IM1600 analyser should have returned a result above the LoL, requiring a series of dilutions to obtain a value within the measurement range, like the results obtained by the Cobas e801 and Alinity i analysers. The diagnosis of complete hydatidiform mole was finally made by combining the patient symptoms with physical examination and other laboratory tests. To eliminate the hydatidiform mole, an ultrasound-guided curettage was performed three days after admission.

HCG assay uses a sandwich method involving two hCG beta-subunit specific antibodies. The first one is an immobilized antibody used as capture antibody, whereas the second one is a labelled-hCG tracer antibody used as the detection antibody. When hCG is present in the sample, it is simultaneously immobilized and labelled, resulting in an immobilized antibody-hCG-tracer sandwich. The amount of immobilized label is measured and directly proportional to the amount of hCG joining the sandwich together. The hCG level in the sample is then obtained after comparison of the amount of tracer signal in the sample to a standard hCG calibration curve.

In case of extremely high concentrations of hCG, which have previously been reported in complete hydatidiform mole, both the capture and the detection antibodies can

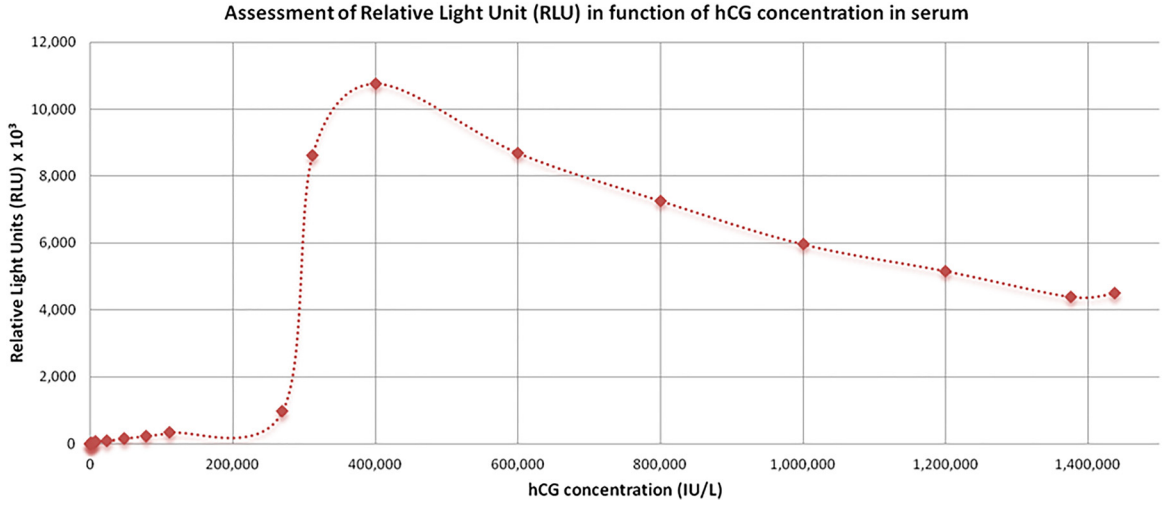

Figure 1: Assessment of relative light unit in function of hCG concentration in serum.

be oversaturated [3]. Non-sandwiched tracer antibodies are washed away with the excess material, decreasing the detected signal [2]. This phenomenon, while rare, is known as "hook effect" and may delay diagnosis and lead to mismanagement of patients.

The measuring interval of most hCG tests is set to the normal pregnancy hCG range at 8–11 weeks of about 25,000 IU/L to 250,000 IU/L [3]. Incidence of the hook effect can be reduced by using a two-step assay with an added washing step, e.g., Abbott Alinity, or by increasing the measurement range of the test, e.g., Roche and Abbott Alinity. At laboratory level, this high-dose effect can be avoided by using dilutions to obtain a concentration within the measurement range of the analysis. In the case of hCG assay, if a diagnosis of gestational trophoblastic disease is suspected, this should be communicated as soon as possible to the laboratory. Thus, the hCG test will be performed on a diluted sample and the hook effect will be avoided.

In conclusion, a good knowledge of the analytical limitations of laboratory tests associated with a complete physical examination is essential to improve patient outcomes and prevent misdiagnosis. To reduce the incidence of the hook effect, analytical procedure has been adapted in our lab and we now systematically verify by dilution each result of hCG stagnating between 500 IU/L and 1,000 IU/L within a period of 7 days.

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