Editorial

David G. Grenache

Progress in understanding the use of human chorionic gonadotropin as a tumor marker

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Immunoassays for the measurement of human chorionic gonadotropin (hCG) have been available for over six decades. Although they were first developed as a test to diagnose pregnancy, other clinical applications emerged as a greater understanding of the biochemical properties and the biological functions of hCG were elucidated. Presently, hCG measurements are also used in the assessment of women with suspected ectopic pregnancy, in conjunction with other biomarkers when screening for fetal aneuploidies and as a tumor marker for a variety of neoplasms. It is important to note that the majority of tests for hCG have not received regulatory approval for clinical uses beyond the detection of pregnancy. Regardless, such “off label” uses are supported by robust evidence and are included in clinical practice guidelines published by many professional societies.

As a tumor marker, hCG is most frequently associated with gestational trophoblastic disease and testicular germ cell tumors. Gestational trophoblastic disease comprises a heterogeneous group of interrelated lesions derived from placental trophoblasts and all trophoblastic tumors secrete hCG. Testicular cancers are histologically differentiated into seminomas and non-seminomatous germ cell tumors (NSGCT). hCG is elevated in 15–20% of men with seminomas and 40–50% of patients with NSGCT [1].

hCG is a molecularly heterogeneous hormone and numerous hCG variants have been identified in blood and urine. These include the biologically active intact hCG (hCG), nicked hCG (hCGn), beta subunit (hCG β), nicked beta subunit (hCG β n) and beta core fragment (hCG β cf). The relative abundance of these molecular forms varies across biological matrix (e.g. blood or urine) and the condition associated with hCG production (e.g. pregnancy or cancer). For example, 20 to 40% of men with seminomas will have elevated concentrations of hCG β alone [2] as will the majority of women with placental site trophoblastic tumor [3].

The heterogeneity of hCG poses an analytical challenge and significant between-method variation in

measured concentrations has been described [4, 5]. This variation is attributed to the use of different reagent antibody pairs, which will determine the specific hCG variants that are detected, and differences in the secondary standards used by manufacturers to calibrate hCG assays. hCG assays are commercially available that detect only dimeric forms of the hormone (hCG and hCGn), dimeric and hCG β /hCG β n forms, and the latter plus hCG β cf [4, 5]. To further complicate matters, the detected variants are frequently not measured in equimolar quantities. In consideration of the biological and analytical complexities, one can appreciate that the use of hCG as a tumor marker is not a straightforward matter and more research is needed.

In this issue of the *Clinical Chemistry and Laboratory Medicine* there are several papers that seek to shed light on precisely this topic. The article by Pretorius et al. describes the answer to a fundamental question: are assays that measure hCG and hCG β (often referred to as total hCG assays) suitable when used for oncology applications [6]? They measured total hCG across four assays using 390 serum samples in which the hCG testing was requested as a tumor marker (65% from males) and a control group of 208 serum samples obtained from pregnant females. The regression slopes obtained from the tumor marker samples for all possible assay pairs were similar to and overlapped with those obtained using the pregnancy samples. However, as has been shown before, the variable regression slopes across assays emphasizes the need to consistently use the same hCG method when performing serial measurements over time. Reassuringly, the rates of outlier samples (those that produced a discordant result in one or more methods) were no different between the tumor marker samples and the pregnancy samples. This is somewhat surprising given the heterogeneity of hCG and the methods that measure it. It is unfortunate that Pretorius and colleagues did not identify the type of malignancy in the vast majority of the tumor marker group as that information would have been a powerful contribution. For example, clinical histories were only provided for cases with a discordant hCG result, two of which were known to have seminoma. One of the

assays used in their report is known to over-estimate hCG β by approximately two-fold yet this assay was not responsible for any of the outliers identified. This is curious and warranting of further investigation. Another criticism of the Pretorius study is provided by Ferraro and Panteghini who, in their Letter to the Editor [7], called Pretorius to task for choosing to define the detection capability of the assays as the limit of blank (rather than the limit of detection) and showing some results below that threshold. In fairness to Pretorius, they acknowledged this in their paper, and it was based on information provided in the product inserts. Indeed, Ungerer and Pretorius make this argument in their rebuttal letter [8].

The use of a highly sensitive hCG assay when the hormone is used as a tumor marker is highlighted by a case report from Ferraro et al. [9]. They describe a man with an unusual testicular neoplasm that was immunohistochemically negative for hCG but for whom serum hCG was 30 IU/L using the Elecsys hCG + β (Roche Diagnostics) assay. During therapy, hCG concentrations decreased to below the limit of detection (0.2 IU/L) but in post-chemotherapy monitoring, hCG concentrations fluctuated between <0.2 and 1.1 IU/L. Those same samples were also analyzed using the Architect Total β -hCG (Abbott Laboratories) assay with a limit of blank of 1.2 IU/L (limit of detection not defined). Whereas 14% of the samples measured by the Elecsys assay were <0.2 IU/L, 59% of the samples measured by the Architect assay were <1.2 IU/L. During surveillance, the detection of a low concentrations of a tumor marker that was previously undetectable may be a biochemical signal of recurrence. However, hCG can also be produced by the pituitary gland in males and females with primary hypogonadism and low serum concentrations in such cases have been documented [10–12]. In this case report, the patient did not have recurrent disease and the fluctuating concentrations of hCG (as determined by the Elecsys) along with elevated serum concentrations of luteinizing hormone and follicle-stimulating hormone and decreased total and free testosterone suggested hCG of pituitary origin. One can speculate that use of the less sensitive Architect assay, would have spared any associated anxiety and/or therapeutic interventions caused by the low hCG concentrations that were detected.

The “optimal” analytical sensitivity for hCG continues to be a topic to be explored and it, among other analytical requirements, is discussed in an opinion paper by Ferraro and Panteghini [13]. In addition to calling for insights to help define optimal analytical sensitivity, the authors also emphasize the need for decision limits (i.e. reference cutoffs) when hCG is used as a tumor marker. Most published hCG decision limits are derived from

healthy, non-pregnant individuals and are specific to a particular method [2, 11, 14]. As these and other studies demonstrate, it is not uncommon to detect low concentrations of hCG if the assay used has high analytical sensitivity. In their article, the authors suggest a more desirable reference population on whom to base decision limits in oncology would be those in whom a malignancy was suspected but were free from disease during clinical evaluation. Finally, the authors also remind us of the importance that laboratorians play in selecting and offering appropriate hCG assays that are fit for the clinical purpose for which they are used. It is difficult for some laboratory professionals and impossible for others to know why a hCG test was requested. In light of this, most would agree that it is essential for hCG assays used in clinical laboratories to be capable of detecting hCG and hCG β . Others have advocated for separate assays that specifically detect hCG and hCG β due to their potential to provide improved diagnostic sensitivity for patients with testicular cancer [15, 16].

While the importance of offering an hCG assay that has clinical utility as a tumor marker is clear, many laboratory professionals lack awareness of the analytical specificity of the hCG assay used in their laboratories. One report identified 9% of laboratories reporting hCG test results as “total hCG” when, in fact, the assays detected only intact hCG and 13% of laboratories reporting results as “intact hCG” when the assay was specific for total hCG [17]. While such reporting errors are unlikely to affect a pregnancy diagnosis, reporting a total hCG result from an intact hCG-specific assay will fail to identify some individuals with malignancy-associated hCG. A contributor to this problem is the manufacturer’s failure to clearly state what hCG variants are detected by their assays.

While our understanding of the analytical and clinical complexities of hCG has broadened, there is much still to be done. There remains an urgent need to educate clinicians and laboratorians regarding the appropriate use and reporting of hCG test results, harmonizing hCG assays and achieving equimolar recognition of hCG variants, and expanding the intended use of hCG tests to include oncological applications are essential. Laboratorians, manufacturers and regulators must work cooperatively to achieve these important goals.

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David G. Grenache, PhD, TriCore Reference Laboratories, 1001 Woodward Place NE, Albuquerque, NM 87102, USA, E-mail: david.grenache@tricore.org