Clinical Chemistry 64:2
270–278 (2018)

Review

Human Chorionic Gonadotropin Assays for Testicular Tumors: Closing the Gap between Clinical and Laboratory Practice

Simona Ferraro,[1*] Chiara Trevisiol,[2] Massimo Gion,[3] and Mauro Panteghini[1]

BACKGROUND: Clinical practice guidelines recommend the measurement of human chorionic gonadotropin (hCG) and/or hCGβ in serum for management of testicular germ cell tumors (GCTs). These guidelines, however, disregard relevant biochemical information on hCG variants to be detected for oncological application. We set out to provide a critical review of the clinical evidence together with a characterization of the selectivity of currently marketed hCG immunoassays, identifying assays suitable for management of GCTs.

CONTENT: Evidence sources in the available literature were critically appraised. Most instances of misdiagnosis and mismanagement of testicular GCTs have been associated with hCG results. According to the clinical evidence, 36% of patients with seminoma show an exclusive hCGβ increase, and 71% of patients with nonseminomatous GCTs (NSGCTs) show an increase of intact hCG and/or hCG + hCGβ, whereas the hCGβ increase in NSGCTs is variable according to the tumor stage and histology.

SUMMARY: hCG + hCGβ assays that display an equimolar recognition of hCG and hCGβ, or at least do not overtly underestimate hCGβ, may be employed for management of testicular GCTs. Assays that underestimate hCGβ are not recommended for oncological application. In addition to the hCG + hCGβ assay in service, an additional assay with broader selectivity for other hCG variants should be considered when false-negative or false-positive results are suspected on the basis of clinical data.
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The global incidence of testicular cancer amounts to 55 000 cases/year, with a higher prevalence in younger Caucasian men *(1)*. In total, 95% of these neoplasms are germ cell tumors (GCTs),[4] including 50% seminomas and 50% nonseminomatous GCTs (NSGCTs). The latter group is further subclassified into embryonal carcinomas, choriocarcinomas, yolk sac tumors, and mature or immature teratomas; however, most NSGCTs are mixed because several histological components are present *(2)*.

The Institute of Medicine defines clinical practice guidelines (CPGs) as "statements that include recommendations, intended to optimize patient care, that are informed by a systematic review of evidence and an assessment of the benefits and harms of alternative care options" *(3)*. Circulating tumor markers (TMs) have a crucial role in decision-making for a limited number of malignancies, including testicular cancer in which human chorionic gonadotropin (hCG), α-fetoprotein, and lactate dehydrogenase have an established clinical application *(4)*. Therefore, CPGs on testicular malignancies are expected to provide recommendations that help clinicians to use these TMs appropriately. Recently, a systematic revision of CPGs in solid tumors has been performed, and recommendations on TMs have been extracted, clustered, and summarized *(5)*; 23 guidance documents were related to testicular cancer and 17 addressed TMs. Most of the revised CPGs, however, do not appear to address TMs properly. Although the CPGs address what TMs should be used, when they should be measured, and what cutoff point should be adopted, they do not consider how TMs should be measured, a critical issue that directly influences the clinical value of TMs.

Although the most authoritative CPGs *(4, 6–8)* recommend the measurement of "hCG" and/or "hCGβ" in serum for the management of testicular GCTs, there is evidence that the test is underused and its results are often

[1] Department of Biomedical and Clinical Sciences "Luigi Sacco," University of Milan, and Clinical Pathology Laboratory, ASST Fatebenefratelli-Sacco, Milan, Italy; [2] Istituto Oncologico Veneto (IOV) – IRCCS, Padua, Italy; [3] Regional Center and Program for Biomarkers, Department of Clinical Pathology and Transfusion Medicine, Venice, Italy.
* Address correspondence to this author at: UOC Patologia Clinica, Ospedale "Luigi Sacco," Via GB Grassi 74, 20157 Milano, Italy. Fax +39-023-904-2766; e-mail simona.ferraro@asst-fbf-sacco.it.
Received April 16, 2017; accepted September 14, 2017.
Previously published online at DOI: 10.1373/clinchem.2017.275263
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[4] Nonstandard abbreviations: GCTs, germ cell tumors; NSGCTs, nonseminomatous GCTs; CPGs, clinical practice guidelines; TMs, tumor markers; hCG, human chorionic gonadotropin; ASCO, American Society of Clinical Oncology; NACB, National Academy of Clinical Biochemistry; hCG-h, hyperglycosylated hCG; IRRs, International Reference Reagents.

misinterpreted *(9, 10)*. The issues surrounding the test request and the result interpretation are several and often neglected, mainly related to the heterogeneity of the measurand and to the difficulty of providing harmonized hCG methods *(11)*. Notably, the "technical" performance of currently available hCG assays for oncology applications has been reported to be the primary cause of patient misclassification *(10, 12)*. A review of the literature raises the following concerns:

(1) the differing histology of testicular GCTs (seminomas or NSGCTs) drives the different tissue expression and the detectability in serum of various hCG variants *(13)*;
(2) an unambiguous nomenclature should be adopted for the most important hCG forms *(14)*;
(3) assays commonly used in the clinical laboratory are validated and approved only for pregnancy and not for cancer management *(10)*.

These 3 concerns provide essential background information to be discussed, shared, and clarified between laboratory professionals and clinicians to enable the most appropriate hCG request, assay selection, and result interpretation. To avoid not making any recommendations, or issuing biased and misleading recommendations, CPGs should therefore include substantial information on hCG variants to be detected in relation to the histological GCT type as well as on the assays theoretically suitable for application in oncology. Among the recently released CPGs recommending measurement of TMs for managing testicular cancer, only the guideline of the American Society of Clinical Oncology (ASCO) *(4)* adopts an unambiguous nomenclature for hCG forms and makes a clear report regarding the main features of the assays needed for appropriate use in oncology; in issuing this guideline ASCO follows the recommendations made in the National Academy of Clinical Biochemistry (NACB)[5] guideline *(15)*. In general, the issue of the selectivity of hCG assays is very complex and deserves more clarification in CPGs, considering the impact of hCG results on the management of patients with suspected or diagnosed testicular GCTs.

The aim of this review is to critically appraise the main evidence regarding hCG assays suitable for detection/monitoring of testicular GCTs, considering the differences between recommendations by CPGs and laboratory practice. Given the complexity of the literature related to the characterization of selectivity of hCG assays as well as the wide heterogeneity of available clinical data on hCG diagnostic performance, a review focused on closing the gap between clinical and laboratory practice in this area may represent a valuable aid in patient management.

hCG FORMS DRIVING DIAGNOSTICS OF TESTICULAR GCTs

The appropriate hCG assay for diagnostics/management of testicular GCTs should account for the histology of the malignancy driving the tissue expression of specific hCG variants as well as for their immunoreactivity in serum *(13)*. Testicular GCTs can be divided into 2 categories expressing different hCG variants. The first group includes choriocarcinoma and testicular malignancies taking on a cytotrophoblast histology (NSGCTs) producing hyperglycosylated hCG (hCG-h) in early and advanced stages *(16, 17)*. The term "hyperglycosylated" refers to the expression of acidic variants of hCG that have complex extensively sialylated terminal carbohydrate antennae. Currently, hCG-h refers to the serum forms only detectable by immunoassays using the B152 monoclonal antibody; it has also been called "invasive trophoblast antigen," although there is no evidence that it has an invasive function or uses any other receptor than the LH/hCG receptor *(14, 18)*. In addition to hCG-h in NSGCTs, nicked hCG, hyperglycosylated nicked hCG, and nicked/nonnicked hCGβ may also demonstrate serum immunoreactivity *(19)*.

The second group of testicular GCTs includes seminomas that take on a nontrophoblastic histology and produce a mixture of hCGβ and hyperglycosylated hCGβ in advanced stages *(20)*. Earlier studies have reported data from both immunohistochemistry and immunoassays in patients with pure seminoma and found a variable incidence (from 7% to 40%) of hCGβ plus hyperglycosylated hCGβ secretion and related serum increase *(21–23)*. What marker was found increased was strictly dependent on the disease stage, and, in fact, almost every cancer in an advanced stage may produce hCGβ forms; a hCGβ increase in serum is generally detectable in approximately 30% of overall cancer cases *(24, 25)*.

Some evidence on the hCG variants detectable in serum of patients with seminomas and NSGCTs can be found in original clinical studies performed using immunoassays for "total hCG" and specific hCG variants (Table 1) *(21, 26–37)*. However, these results should be interpreted with caution since most of the studies are dated and, consequently, the evidence cannot be directly transferred to current commercially available assays; furthermore, these study results have not been revised according to the selectivity of hCG assays, more recently characterized by the use of WHO International Reference Reagents (IRRs) *(38–40)*.

For pure seminomas, Mann and Siddle found increased "total hCG" concentrations in serum of 12% of studied patients by use of an hCG + hCGβ RIA, standardized against the WHO International Standard 75/

[5] The National Academy of Clinical Biochemistry (NACB) is now the AACC Academy.

Table 1. Synopsis of studies on patients with diagnosis of seminoma or nonseminomatous germ cell tumors reporting detected human chorionic gonadotropin forms and rates of positivity.[a]

Authors (year)	Histology	Method	Detected hCG variant(s)	Positivity rate (total number of patients)
Cochran (1976) *(21)*	Seminoma	RIA	hCG (?)[b]	10% (20)
Mann and Siddle (1988) *(26)*		RIA	hCG + hCGβ	12% (349)
		IRMA	hCG	37% (19)
		IRMA	hCGβ	37% (19)
Ruther et al. (1994) *(27)*		IRMA	hCGβ (?)	30% (106)
Madersbacher et al. (1992) *(29)*		ELISA	hCG	15% (33)
		ELISA	hCGβ	20% (33)
		ELISA	hCG + hCGβ	25% (33)
Germa-Lluch et al. (2002) *(30)*		Not available	hCGβ (?)	21% (533)
Lempiäinen et al. (2008) *(31)*		IFMA	hCG	17% (42)
		IFMA	hCGβ	52% (42)
Saller et al. (1990) *(32)*		IRMA	hCG	20% (51)
		IRMA	hCGβ	30% (51)
		IRMA	hCG + hCGβ	48% (51)
Hoshi et al. (2000) *(33)*		IFMA	hCG	50% (54)
		ELISA	hCGβ	83% (54)
Lempiäinen et al. (2012) *(34)*		IFMA	hCG	0% (29)
		IFMA	hCGβ	45% (29)
		IFMA	hCG-h	1% (29)
Lempiäinen et al. (2014) *(35)*		IFMA	hCGβ	52% (34)
Javadpour et al. (1978) *(36)*		RIA	hCGβ (?)	8% (130)
Javadpour et al. (1978) *(37)*		RIA	hCGβ (?)	5% (60)
Mann and Karl (1983) *(28)*	NSGCT	RIA	hCG	60% (56)
		RIA	hCGβ	23% (56)
Madersbacher et al. (1992) *(29)*		RIA	hCG	60% (76)
		ELISA	hCG	58% (76)
		ELISA	hCGβ	40% (76)
		ELISA	hCG + hCβ	59% (76)
Germa-Lluch et al. (2002) *(30)*		Not available	hCGβ *or* hCG + hCGβ (?)	53% (957)
Lempiäinen et al. (2008) *(31)*		IFMA	hCGβ	78% (51)
		IFMA	hCG	73% (51)
Saller et al. (1990) *(32)*		IRMA	hCG + hCGβ	96% (55)
Hoshi et al. (2000) *(33)*		IFMA	hCG	82% (74)
		ELISA	hCGβ	74% (74)
Lempiäinen et al. (2012) *(34)*		IFMA	hCG	74% (38)
		IFMA	hCGβ	79% (38)
		IFMA	hCG-h	74% (38)
Lempiäinen et al. (2014) *(35)*		IFMA	hCG	71% (73)
Javadpour et al. (1978) *(37)*		RIA	hCGβ (?)	90% (400)

[a] Original studies reported in the table were retrieved by a systematic review of literature on Medline and Embase databases by using the key terms "testicular/testis cancer human chorionic gonadotropin" and selected if reporting hCG results in patients with testicular cancer. The search spanned from 1975 to 2016.
[b] The question mark indicates that the reported hCG form does not comply with the declared assay selectivity or the hCG nomenclature is ambiguous.

537 *(26)*. In 19 patients, hCG and hCGβ were also measured by monoclonal immunoradiometric assays, which showed increased concentrations of either marker in 37% of subjects. In addition, there was evidence that hCGβ concentrations increased according to the disease stage *(26)*. A further study, using a similar immunoradiometric assays method, confirmed an hCGβ increase in 30% of seminoma patients *(27)*.

In a previous study, with use of an inhouse RIA, the authors investigated the secretion of hCG, hCGα, and hCGβ in 56 NSGCTs patients and found increased hCG concentrations in 60%, whereas only 23% and 15% showed an increase of hCGβ and hCGα, respectively *(28)*. A similar study evaluated 109 testicular cancer patients (33 seminomas and 76 NSGCTs) and found a similar rate of positive hCG and hCG + hCGβ results (approximately 60%) in NSGCTs *(29)*. In seminoma patients, hCG, hCGβ, and hCG + hCGβ had almost equal but lower prevalence (15%–25%) *(29)*. After publishing similar data, Saller et al. suggested the clinical utility of detecting hCG and hCGβ in seminomas, by either 2 distinct assays or a single hCG + hCGβ assay *(32)*.

Retrieving data from 1994 to 2001, Germa-Lluch et al. reported on 1490 patients with testicular GCTs (64% NSGCTs and 36% seminomas) from 55 hospitals belonging to the Spanish Germ Cell Cancer Group *(30)*. The authors found a positive result for "β hCG" in 21% of seminomas and 53% of NSGCTs, respectively. Although this survey was later cited by several prominent authors and by the NACB guideline *(15)*, these results should be considered with caution since no data were reported on the employed assays.

Hoshi et al. measured hCG and hCGβ in 54 patients with seminoma and 74 with NSGCTs, concluding that in seminoma patients a single measurement of hCGβ was more effective than a single measurement of hCG because hCGβ was positive in 83% vs hCG positive in 50% of cases *(33)*. Similar conclusions were drawn by Lempiäinen et al. *(31)*, for whom: a) in seminomas, the single measurement of hCGβ was more useful than hCG; b) in NSGCTs, increases of either hCG and hCGβ were mostly concordant (approximately 75% of cases).

In a further study, Lempiäinen et al. evaluated whether the separate detection of hCG-h and hCGβ, both measured by immunofluorometric assays, may provide diagnostic and prognostic information additional to that of hCG *(34)*. Because no international standard is available for hCG-h, a provisional standard (i.e., hCG-h from the JEG-3 choriocarcinoma cell line medium) was used to check the assay selectivity. Lempiäinen et al. concluded that although hCG in patients with NSGCTs was mainly hyperglycosylated, the determination of hCG-h in serum did not provide clinical information additional to that obtained by conventional hCG assays that also recognize hCG-h; by contrast the separate measurement of hCGβ enhanced the detection of relapse and improved the diagnosis of seminomatous tumors *(34)*. The most recent study by the same group has investigated the relationship between the immunohistochemical expression and serum concentrations of hCG, hCG-h, and hCGβ in testicular tumors *(35)*. In NSGCTs, these investigators found that serum concentrations of hCG, hCGβ, and hCG-h were well correlated with tissue staining of the same hCG forms. Seminoma tissue expressed mainly hCGβ, whereas hCG and hCG-h were rarely and never expressed, respectively. Notably, in patients with seminomas, serum concentrations of hCGβ did not correlate with tissue expression, and the increase of hCGβ in serum was far more common (52%) than positive tissue staining (7%) *(35)*.

The evidence from available clinical studies listed in Table 1 shows a wide heterogeneity in performances of hCG and hCGβ measurements, even when simply reported as percentage of positive results in NSGCTs and seminomas. This is likely dependent on the heterogeneity of the evaluated patient populations, with differences in the prevalence of early/late stages within case series, and in the selectivity of the employed assays. Trying to summarize this evidence, we can argue that:

(1) on average, 36% of patients with seminomas show an isolated hCGβ increase, whereas a joined hCG and hCGβ increase cannot be reliably defined;
(2) on average, 71% of patients with NSGCTs show an increase of intact hCG and/or hCG + hCGβ, whereas the hCGβ increase is variable according to the stage of the disease;
(3) increased tissue expression and increases of hCG and hCGβ in serum are comparable only in NSGCTs.

A GUIDE TO SELECT THE APPROPRIATE hCG ASSAY FOR TESTICULAR GCTs

Because no hCG assay currently has regulatory approval for cancer diagnostics, it is extremely important to define exactly the analytical specificity of commercially available hCG immunoassays to gain a formal validation in this clinical framework and to establish the reliability and transferability of the available clinical evidence.

An exhaustive description of the measured hCG variants and the extent to which they are recognized by different assays may be done reliably only by use of WHO IRRs. Up to 2003, the essentially identical 3rd and 4th WHO international standards hCG 75/537 and 75/589 and the 1st International Reagent Preparation 75/551 for hCGβ and 75/569 for hCGα were available. These standards were relatively impure mixtures of hCG, and their quantities were assigned in units on the basis of bioactivity (hCG) or in mass concentrations, in the case of α and β subunits *(41)*. Considering that the obtained concentrations for hCG and its subunits in different materials were not directly related and that other clinically relevant forms lack international standards, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) created a working group to prepare highly purified IRRs for 6 hCG forms, including intact hCG (IRR 99/688), free hCGβ (IRR 99/650), nicked hCG (IRR 99/642) and hCGβ (IRR 99/692), hCGβ

core fragment (IRR 99/708), and hCGα (IRR 99/720) *(42, 43)*.

Since the release of these IRRs, studies have been published to establish the comparability of the 4th WHO international standard hCG 75/589 with the IRR 99/688 and to characterize the selectivity of different available assays by the recovery of newly released IRRs. Sturgeon et al. *(39)* reported results for 4 automated assays measuring intact hCG (Perkin-Elmer AutoDelfia, BioMérieux Vidas, Dade-Behring Dimension, and Roche Elecsys Intact hCG), for 6 automated assays measuring hCG + hCGβ (Abbott Architect, Abbott AxSYM, Abbott IMx, Beckman Access, Siemens Centaur, and Tosoh AIA Total), and for 4 automated assays (Roche Elecsys Total hCG, Siemens Immulite, Siemens Immulite 2000, and Ortho Vitros ECi) and 2 inhouse RIAs measuring hCG + hCGβ + hCGβ core fragments. The recognition of different hCG variants was reported in molar terms, with the results expressed as percent ratio relative to the recognition of hCG IRR 99/688 for each assay. With the recognition of intact hCG, all assays were reported to detect accurately the 75/589 standard against which they had been calibrated. Recovery of IRR 99/688 was found to be on average approximately110–115% for Beckman Access, Siemens Centaur, and hCG + hCGβ Abbott Architect, and far higher for the other assays including RIA. These increased recoveries reflected the higher purity of the IRRs.

With recognition of hCGβ (IRR 99/650) vs hCG (IRR 99/688), a great interassay variability was shown, with 9 assays markedly overestimating hCGβ (in average, +132%). The recognition of nicked hCGβ (IRR 99/692) ranged from 46% to 156%, giving approximately 100% only for Roche Elecsys and Siemens Immulite. For oncological application, however, it is relevant to observe that the variability in nicked hCG recognition is not a major problem.

A further study, performed by Harvey et al. *(40)*, confirmed the results on the recovery of hCG and different forms, reporting a recognition of hCGβ quite similar to that of intact hCG (range: 107%–123%) for most assays, except for the Beckman Access assay which exhibited an overestimate by 2.3-fold, and for the 2 RIAs, which significantly underestimated hCGβ. Interestingly, this study also measured samples of patients with testicular GCTs, highlighting that the interassay variability for detection of hCG variants is greater for hCGβ, and this was recognized as the main cause of discrepancies among assays measuring hCG + hCGβ *(40)*.

Whittington et al. *(38)* performed a study similar to that of Sturgeon et al., but reported lower recoveries for hCGβ. Seven hCG assays detected hCGβ, but only 4 showed equimolar detection of hCGβ vs hCG. One assay (Beckman DxL Total βhCG) overestimated hCGβ, 2 assays (Vitros ECi Total β-hCG II and AIA 1800 ST Total β-hCG) underestimated it, and the Dimension RxL hCG assay was unable to detect it, in accordance with the assay design.

The studies by Cole et al. *(19, 44, 45)* require separate consideration, because this research group tested the recovery of commercial measuring systems by using purified preparations different from WHO IRRs *(46)*. Indeed, their obtained results were substantially different from those reported by previous authors. In the more recent study *(45)*, the authors characterized 12 types of automated hCG assays, reporting on the recoveries of different hCG variants, by supplementing serum and urine samples with the purified standards mentioned above. Only the Siemens Immulite 2000 assay gave consistently acceptable results for all hCG variants and was recommended as the assay of choice for diagnosing and monitoring trophoblastic disease and nontrophoblastic cancer and, more generally, for all applications requiring recognition of both intact hCG and hCG metabolic products *(45)*. Surprisingly, 8 out of 12 assays failed to detect hCGβ appropriately; the authors' conclusion was that, in terms of selectivity for hCG variants, "there was no significant improvement since the 1970s when the manual 'hCGβ' RIA accurately detecting all variants was employed." It should be noted that the quality of Cole's work has been questioned by some authoritative journals owing to violations of scientific publishing standards and the presence of methodologic flaws *(47)*.

When considering all the evidence on the analytical selectivity of commercial hCG immunoassays, the variability of recoveries of different hCG forms undoubtedly reflects differences in assay design and in selected antibodies. No assay appears to be close to the equimolar recognition of all hCG forms *(39)*. The large between-assay variation observed for detection of hCGβ is likely to provide wide discrepancies in hCG results from sera of patients with cancers *(48)*. A further experimental proof of wide discrepancies and of large variation in hCG results among the most popular assays detecting "total hCG" can be derived from proficiency test reports, confirming that the sole addition of hCGβ unexpectedly increases the variability of results *(10, 49, 50)*.

Acknowledging the assay design as the most critical point, in 2013 the International Society of Oncology and Biomarkers Tissue Differentiation recommended for oncology purposes the use of hCG + hCGβ immunoassays with broad selectivity, with use of monoclonal antibodies against the β1 epitope as a capture antibody combined with a detector antibody recognizing β2 or β4 epitopes *(14)*. However, hCG assays built on this design are not yet commercially available, and consequently the recognition of more clinically relevant hCG variants on equimolar basis is currently out of reach *(40)*. By applying a pragmatic approach, some authors have recommended as a minimum requirement for oncology appli-

cation the use of an assay characterized by an equal detection of hCG and hCGβ, considering that trophoblastic tumors produce intact hCG and that hCGβ may predominate as well, frequently being the only form in the serum of patients with GCTs *(32, 42, 51)*. With the use of this simple guidance, out of the 4 assays (Immulite 2000 hCG, Elecsys 2010 hCG+β, Advia Centaur Total hCG, and Architect Total β-hCG) identified by Whittington et al. *(38)* in their study, only 2 (Advia Centaur and Architect) were confirmed to be close to the goal for equimolarity by Sturgeon et al. and Harvey et al. *(39, 40)*.The Advia Centaur can produce falsely decreased hCG results when hCGβ is very high, at concentrations substantially greater than those typically observed in malignancies *(52)*. These conclusions disagree with those of Cole et al. quoted above, reporting Immulite 2000 as the only assay useful in oncology *(19, 44, 45)*. This is an important practical point to be discussed, since several studies have considered the Immulite assay as the "reference method" in this framework and have validated other assays by performing head-to-head comparisons with it on serum samples from patients with GCTs *(53)*. An additional point of practicality concerns the use of RIAs for measuring hCG in testicular GCT management, and RIA methods are often preferentially chosen by hCG reference centers *(45)*. These were originally reported as the only assays satisfying the goal of equimolar recognition of all hCG forms, but more recent studies employing WHO IRRs have demonstrated their significant underestimation of hCGβ *(40)*. All authors agree on banning in oncology the use of assays not detecting hCGβ (Perkin-Elmer AutoDelfia, BioMérieux Vidas, Dade-Behring Dimension, Roche Elecsys Intact hCG, Dimension RxL hCG) and on using the same assay for disease monitoring in the same patient, as the wide variability in hCG + hCGβ results among assays places patients at risk for mismanagement *(39, 40, 52, 54)*. The lack of harmonization across hCG assays remains a critical issue, at least until comparative epitope mapping procedures suggested to improve comparability of results have been applied *(55)*.

WHAT IS THE RIGHT hCG ASSAY FOR TESTICULAR GCTs?

We previously mentioned the suggestion by some authoritative sources to use only assays characterized by an equal detection of intact hCG and hCGβ (the so-called "total hCG" assays) for oncology application. Other authors have dissented from this proposal in favor of separate determinations of intact hCG and hCGβ *(34, 42, 48)*. They recommended applying direct hCGβ measurement for differential diagnosis and monitoring of seminomas and as an aid for diagnosis of nontrophoblastic tumors, which release, in 30–60% of cases, hCGβ but not intact hCG *(54)*. They argued that the upper reference limit for hCG + hCGβ concentrations in serum is higher than that for hCGβ alone, and thus tumors causing only a moderate increase in serum hCGβ might not be detectable by an assay measuring "total hCG" *(26, 33, 34, 42, 54)*. Lempiäinen et al. *(34)* showed in their case series that the use of the assay measuring hCG + hCGβ would have missed all seminoma patients (45% of total) having increased concentration of hCGβ and approximately 5% of marker-positive NSGCTs. Additionally, 1 seminoma patient and 5 NSGCT patients with a relapse after complete remission had increased concentrations of hCGβ with a concentration of "total hCG" within the reference interval *(34)*. Accordingly, if the main aim is to facilitate the earlier detection of a tumor relapse, the specific measurement of hCGβ should be promoted, with use of assays characterized by a low limit of detection *(42)*.

Summarizing different positions, Glenn D. Braunstein named "splitters" those authors who prefer determining intact hCG and hCGβ separately, whereas "lumpers" support the "one-size-fits-all" concept, which is the use of a single assay that measures hCG + hCGβ, ideally in equimolar quantities *(54)*. Braunstein commented that there is no definitive evidence on the cost-effectiveness of using multiple assays vs an all-inclusive single assay, although he advised on the need to choose assays with broader selectivity *(54)*. The failure to know the selectivity of hCG assays toward different hCG forms, related to the lack of information in the manufacturers' product inserts, is well recognized *(10)*. An additional source of errors in reporting patient results concerns the nomenclature adopted for hCG variants recognized by the assays, which is confusing and in some cases misleading. The International Society of Oncology and Biomarkers has recommended the use of "hCG" for the bioactive intact αβ-heterodimer, "hCGβ" for the intact noncombined free hCGβ-subunit (hCGβ aa1–145), and "hCGn" for defining the nicked αβ heterodimer (nicks in the hCGβ aa44–48 region) *(14)*.

Among the current CPGs on management of testicular GCTs, 4 address the hCG measurement *(4, 6–8)*, but only the ASCO CPG clearly recommend the use of double-antibody immunometric assays that measure "total hCG" (i.e., intact αβ heterodimer plus free hCGβ monomer) *(4)*. ASCO refers to the NACB guideline in declaring the lack of evidence for considering the measurement of hCGβ alone to improve testicular cancer detection *(15)*. To pragmatically form a conclusion on the selection of the most suitable hCG + hCGβ assays for use in oncology, after critically appraising the main evidence on assay selectivity present in the literature and summarized in this review, we can argue that at least 4 currently marketed immunoassays (Immulite 2000 hCG, Elecsys 2010 hCG+β, Advia Centaur Total hCG, and Architect Total β-hCG) have broad selectivity and

analytical sensitivity high enough to suitably measure "total hCG" in oncology.

Falsely increased hCG concentrations in serum of patients with testicular GCTs, due to analytical interferences (e.g., heterophile antibodies) or associated clinical conditions (e.g., hypogonadism with increased hCG production by the pituitary gland, tumor lysis induced by chemotherapy), have been reported *(56–63)*. Those conditions that can wrongly influence hCG results should be considered as potential causes of misdiagnosis and mismanagement of testicular GCTs *(64–66)*.

CONCLUDING REMARKS

Although the hCG test is recommended by authoritative CPGs for management of testicular GCTs, both the NACB and the European Group on Tumor Markers guidelines emphasize that this application should be considered off-label for those methods designed to detect the most clinically relevant hCG variants, and require that the employed assay must be declared on the laboratory report *(15, 67)*. However, we have doubt that this information, once explicitly available, can effectively help clinicians to correctly interpret marker results, since knowledge of the assay specificity is generally not available from manufacturers. Nevertheless, laboratory professionals, who are responsible for test procedures and corresponding results, should be aware of the heterogeneity of hCG assays and the selectivity of the method in service *(68)*. Knowledge of the selectivity of the employed commercial hCG immunoassay will help improve the reliability of the clinical interpretation of results and directly affect patients' outcomes *(12, 58, 63)*.

Thus, hCG + hCGβ assays reported with a nearly equimolar recognition of both intact hCG and hCGβ monomer, or at least not showing an overt underestimation of hCGβ, may be used for the management of testicular GCTs. Assays not accurately detecting hCGβ should not be used in oncology. In addition to the "total hCG" assay in service, an extra assay with broader selectivity for other hCG variants should be considered when: a) false-negative hCG results are suspected in relation to the expression of modified cancer-related variants, and/or b) positive hCG results do not comply with clinical data. Finally, in patient monitoring it is crucial to use the same assay because of the still high interassay variability *(69)*.

Clinicians may apply optimal standards for patient care when clinical laboratories offer high quality measurements, which are suitable for clinical application *(70)*. The present study has discussed the gap in the translation of relevant information about the characteristics of hCG assays for use in testicular tumors into clinical practice. We believe that only a multidisciplinary approach can help to focus on important laboratory-related items that can influence healthcare outcomes *(71)*. The issues we have raised highlight the critical need for laboratory expertise when drafting CPGs involving the use of TM tests in GCTs. On the other hand, it is time that regulatory agencies require manufacturers providing commercial kits for hCG determination to declare the selectivity of employed antibodies for clinically relevant hCG variants and the specificity of the antibody combination as a prerequisite for the market clearance of assays.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

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