Editorial

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The clinical value of assessing the inter-method bias: the lesson from prostate specific antigen measurement

https://doi.org/10.1515/cclm-2021-1125

The clinical value of a laboratory test has to be ascertained on the basis of its impact on patient management [1]. Consequently, surveillance of the analytical performances of the test results (i.e., precision and trueness) may be enough if clinicians use results obtained by the same assay or by different assays known to be "sufficiently" harmonized. Conversely, when clinicians are unaware to use marker results from different assays whose harmonization appears to be suboptimal, as has been reported for total prostate specific antigen (tPSA) measurement, further steps should be taken to pursue the appropriate interpretation of the results and consequently improve the clinical laboratory stewardship [2, 3].

This issue becomes relevant when one considers that only ~30–40% of the biopsies performed by the urologists at a clinical center in outpatients are related to a tPSA result performed with the same method used in the laboratory of the same referral clinical center [4]. Therefore, clinicians are often unaware of using heterogeneous tPSA results to offer biopsy to outpatients at increased risk of high-grade disease, to promote active surveillance of slow-growing cancers, and to run effective rescreening programs [5]. During COVID-19 pandemic this situation has spread for all tumor markers (TMs) since clinicians of several first-line hospitals have experienced the challenging malignancy monitoring in outpatients who had to refer to other different laboratories and likely to different methods for TMs measurement to continue cancer surveillance through serial evaluation of TMs circulating concentrations [6, 7]. The limited access to healthcare services of several first-line hospitals facing this pandemic has caused a drop in the rate of TMs ordering and determination by ~30–50% and a consequent migration of outpatients to other clinical laboratories for TM monitoring was unavoidable [6, 7]. In particular, we recorded −48.5% and −28.9% of the determinations comparing the absolute number of tPSA requests in the pre-pandemic semester vs. the semester of the first outbreak (1st March–31st August 2019 vs. 1st March–31st August 2020) and then vs. the semester of the second outbreak (1st March–31st August 2019 vs. 1st March–31st August 2021). This undoubtedly contrasts with the recommendation of several clinical guidelines on cancer surveillance, including prostate cancer, which state that the monitoring of the disease based on serial determination of TMs should be performed using the same method due to the poor inter-method agreement [5].

To this regard, the confusion and the disappointment of the clinicians who have to deal with "disparate results obtained from different manufacturers" for decision making, when the laboratory changes the analytical methods for measuring TMs such as prostate specific antigen (PSA) that play a crucial role in clinical decision making, have been well described [8]. Some authors who have experimented switching to an alternative tPSA assay have suggested dual reporting of the results of both methods to prevent inter-method bias between the replaced and the new method from influencing the clinical decision [8]. However, from a cost-benefit perspective, dual reporting has been limited to clinically relevant concentration ranges (i.e., around the decision cut-off) and to a minimum time duration so that clinicians could re-establish baseline values [9].

In such a situation, however, it should be relevant to actually define whether the reported 11% inter-method bias of the tPSA measurement might be tolerated or whether the method-dependency of tPSA results may affect the patients' outcomes (i.e., increase the rate of undue biopsies, slow-growing cancers treated, patients undergoing rescreening programs), causing an unfavorable risk-benefit ratio of the tPSA based-screening strategy [2]. Therefore, assessing the effect of the inter-method bias on the clinical

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outcome is undoubtedly a valuable option for markers such as PSA that play a central role in decision making [10]. This should account that the optimal goal for the ratio between the number of detected advanced cancers and of performed biopsies to fulfill in the referral context is 1:3 [4]. The main criticism in this framework is the use of established sharp cut-off point, since: a) there is still a heated debate about the shift from 4.0 to 3.1 ng/mL following recalibration to World Health Organization (WHO) International Standard (IS) 96/670 and b) it makes diagnostic decisions highly dependent on inter-method bias, likely non proportional/linear [5]. These methodological issues further endorsed the shift from the use of a fixed diagnostic decision limit to the introduction of tPSA based-risk thresholds for advanced cancer and biopsy referral [4, 5]. From this perspective, the evaluation and study of the pattern of the inter-method bias becomes challenging, as, to be pragmatic, there is a need to establish if tPSA based-risk thresholds for advanced cancer, obtained from one method and estimated according to well calibrated risk prediction models, can be reliably converted into the corresponding concentrations assayed by the other methods [4]. Considering the ongoing studies it has been criticized that the comparison of PSA methods might require a higher number of clinical samples, well distributed over the entire range of measurements, even though this might imply the use of different analytical runs [3]. In order to minimize the effect of experimental factors on bias variation, close control of the experimental conditions and analytical stability of the automated methods is mandatory. In addition to the study design and sample size we must pay the utmost attention to the case mix, since an adequate number of samples from patients with advanced cancers, glandular inflammation, and benign hyperplasia has to be encountered. Indeed, we have to account that in these cases the assays may give "disparate results" due to the antibodies employed according to the heterogeneity of the antigens. These cases are likely to increase in the referral with respect to the screening context which is generally considered to retrieve samples for performing the comparisons between methods [2]. In agreement with recent data, several authors have reported discordant outlying results on a limited group of samples (whose proportion has not changed with the recalibration to the WHO IS) and this has been ascribed

Table 1: Characteristics of all measuring systems and of the antibodies used for total PSA automated immunoassays quoted in refs. [1, 2].

Manufacturer	Platform	Method principle	Commercial name	Capture antibody[a]	Tracer antibody[a]	Stated traceability
Abbott Diagnostics	Alinity i	CMIA	Total PSA serum[b]	Mouse monoclonal H50, ISOBM code 57, Epitope group 3	Mouse monoclonal H117, ISOBM code 56, Epitope group 6	WHO IS 96/670
Beckman Coulter	Access Dxl	CLIA	Access Hybritech PSA	Mouse monoclonal PSA399, ISOBM code 84, Epitope group 3	Mouse monoclonal PSM773, ISOBM code 86, Epitope group 5	WHO IS 96/670
Roche Diagnostics	Cobas e801	ECLIA	Elecsys total PSA	**[b]Mouse monoclonal PSA36, ISOBM code 75, Epitope group 6b**	**[c]Mouse monoclonal PSA66, ISOBM code 74, Epitope group 4b**	WHO IS 96/670
Ortho Clinical Diagnostics	Ortho Vitros XT 7600	CLIA	VITROS total PSA II	**[b]Mouse monoclonal PSA36, ISOBM code 75, Epitope group 6b**	**[d]Mouse monoclonal PSA66, ISOBM code 74, Epitope group 4b**	WHO IS 96/670
Siemens Healthcare Diagnostics	Atellica IM	CLIA	Atellica IM Prostate specific antigen (PSA)	Mouse monoclonal 1F7, ISOBM code not reported, Epitope group 2	Polyclonal goat MP2, ISOBM code 62, Not classified into PSA epitopes groups	WHO IS 96/670

WHO, World Health Organization; IS, International Standard; CMIA, chemiluminescent microparticle immunoassay; PSA, prostate specific antigen; tPSA, total prostate specific antigen; ISOBM, International Society of Oncology and Biomarkers; CLIA, chemiluminescence paramagnetic particle immunoassay; ECLIA, electrochemiluminescent immunoassay. [a]Information about antibodies selectivity and classification were provided by the corresponding manufacturer. The ISOBM antibody classification for all assays excepted from Ortho Clinical Diagnostics (here added) has been reported in ref. [2]. In bold it is highlighted the sameness of antibodies used by Ortho and Roche immunoassays. [b]Biotinylated mouse monoclonal PSA36. [c]PSA 66 labeled with a ruthenium complex. [d]PSA 66 labeled with horseradish peroxidase.

to the differences in the recognition of the various circulating PSA forms [2]. According to the cumulated evidence, we think that the observation that some methods such as Ortho and Roche assays are "more in line with the Hybritech calibration" than others that have undergone WHO calibration should be cautionary considered [2, 3]. It might be associated to the difference (or, conversely, similarities) in the antibodies employed by some assays vs. others described in Table 1 (e.g., Abbott vs. Ortho and Roche assays, these latters having the same capture and detection antibodies and differing only in the tracer molecule). Notably the bias is highest in the patients with highest tPSA values who have a highest probability of harboring advanced cancers and thus of releasing different serological forms that can be variably recognized by the antibodies of the different assays [2, 5].

All the previous criticisms highlight the need of broadening result report accuracy to diagnostic accuracy, as an accurate diagnosis is a prerequisite for an optimum treatment decision [1].

To pragmatically fulfill this aim the use of appropriate statistical methods to assess the agreement of the assays according to an appropriate study design is crucial to evaluate the possibility to introduce feasible and pragmatic options (i.e., method dependent cut-off points/risk thresholds) to avoid that diagnostic decisions could be strongly affected by the bias between assays.

Research funding: The authors received no funding for this work.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

References

1. Plebani M. Clinical laboratory: bigger is not always better. Diagnosis (Berl) 2018;5:41–6.
2. Ferraro S, Bussetti M, Rizzardi S, Braga F, Panteghini M. Verification of harmonization of serum total and free prostate-specific antigen (PSA) measurements and implications for medical decisions. Clin Chem 2021;67:543–53.
3. Kavsak PA, Hotte SJ. A large number of fresh samples and a wide range of total prostate-specific antigen (tPSA) concentrations is important to detect differences in PSA methods. Clin Chem 2021; 67:1155–7.
4. Ferraro S, Bussetti M, Bassani N, Rossi RS, Incarbone GP, Bianchi F, et al. Definition of outcome-based prostate-specific antigen (PSA) thresholds for advanced prostate cancer risk prediction. Cancers 2021;13:3381–95.
5. Ferraro S, Bussetti M, Panteghini M. Serum prostate specific antigen (PSA) testing for early detection of prostate cancer: managing the gap between clinical and laboratory practice. Clin Chem 2021;67:602–9.
6. Durant JST, Peaper DR, Ferguson D, Schulz WL. Impact of COVID-19 pandemic on laboratory utilization. J Appl Lab Med 2020;5: 1194–205.
7. van de Haar J, Hoes LR, Coles CE, Seamon K, Fröhling S, Jäger D, et al. Caring for patients with cancer in the COVID-19 era. Nat Med 2020;26:665–71.
8. Rutledge AC, Pond GR, Hotte SJ, Kavsak PA. Assessing the necessity of including a crossover period with dual reporting when changing total prostate-specific antigen methods. Clin Biochem 2014;47:897–900.
9. Kittanakom S, Donohue V, Beattie J, Clark L, Kavsak PA. The importance of tumour marker dual reporting during method transition: PSA high-dose hook effect detected. Clin Biochem 2018;61:45–6.
10. Smith AF, Shinkins B, Hall PS, Hulme CT, Messenger MP. Toward a framework for outcome-based analytical performance specifications: a methodology review of indirect methods for evaluating the impact of measurement uncertainty on clinical outcomes. Clin Chem 2019;65:1363–74.