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Measurement Uncertainty – Based Assessment of Laboratory Tests for Ovarian Cancer-Related Tumor Markers

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ABSTRACT: Measurement uncertainty is an intrinsic parameter showing the dispersion of numerical values. Cancer antigen 125 (CA 125) and human epididymis protein 4 (HE4), along with the risk of ovarian malignancy algorithm (ROMA) value, are widely used tumor markers to determine the risk of ovarian cancer. Although predefined cutoffs interpret tumor markers, their intrinsic uncertainties produce an ambiguous range for risk assessment. This study assessed the measurement uncertainty of CA 125, HE4, and ROMA values to determine reliable reporting ranges for these markers, which suggest the optimal reporting strategy. Results indicated that the reliable reporting range was below 32.5 or above 37.9 U/mL for CA 125, below 64.5 or above 76.6 pmol/L for premenopausal HE4, below 128.9 or above 153.1 pmol/L for postmenopausal HE4, below 6.7 or above 8.4% for premenopausal ROMA value, and below 22.7 or above 28.6% for postmenopausal ROMA value. Analysis of real patient data revealed that approximately 4–6% of test results fell outside of the reliable reporting range, underscoring the importance of cautious interpretation. The findings suggested that incorporating measurement uncertainty into clinical practice could enhance the reliability of tumor marker results, potentially improving patient management and decision-making.

KEYWORDS: Biochemistry, Medical Biochemistry, Tumor Marker, Uncertainty Measurement, Reliable Reporting Range.

Introduction

Measurement uncertainty (MU) is a non-negative value that indicates the dispersion of the data and is a commonly used concept in various industries and engineering fields. Although most of the information provided by clinical laboratories has a quantitative character, the importance of uncertainty has been raised relatively recently. In practice, it represents the range within which the true value of the measurand is expected to fall, given a specified level of confidence. While the word "uncertainty" is often associated with doubt in everyday language, in the context of medical laboratories, it instead conveys greater assurance about the reliability of a reported measurement. Uncertainty estimation can be performed using two approaches: the top-down and bottom-up methods. The Guide to the Expression of Uncertainty in Measurement (GUM), published in 1996, states that the bottom-up approach is the standard method for estimating MU, which involves the identification of all sources of uncertainty in the measurement procedures, estimation of their magnitudes, and calculation of the combined uncertainty according to the law of error propagation.[1,2] Meanwhile, the MU guideline for laboratory medicine recommends that the top-down approach using imprecision data obtained from internal quality control (IQC) results is practical and particularly well-suited to closed measurement systems (instruments, calibrators, and reagents from the same vendor).[3]

Globally, ovarian cancer is the eighth most common cancer in women and the second most common gynecologic cancer in the United States, and ovarian cancer causes more deaths than any other cancer of the female reproductive system.[4] Its five-year survival rate is about 50%, and it is markedly influenced by stage.[5] That is, early detection is very important for the better management of patients. In this regard, the importance of useful biomarkers of ovarian cancer has been stressed for several decades. CA 125 (cancer antigen 125) is a well-known biomarker for ovarian cancer and has been widely used for monitoring and recurrence detection of ovarian cancer.[6,7] Also new ovarian cancer marker, HE4 (human epididymis protein 4), has been developed and introduced into clinical practice.[8-11]

The risk of ovarian malignancy algorithm (ROMA) value is another valuable diagnostic marker to predict the risk of epithelial ovarian cancer, with higher overall accuracy than HE4 and CA-125, and is characterized as having higher specificity and negative predictive value.[12] ROMA value is calculated from the predictive index (PI) derived from CA 125 and HE4 in premenopausal and postmenopausal status and shows the percent risk for epithelial ovarian cancer.[13,14]

Most of the tumor markers are interpreted based on cutoffs discriminating low risk vs. high risk, or reference intervals derived from the healthy population.[15] However, those cutoffs are a relative compromise with intrinsic uncertainty, rendering the interpretation of test results prone to errors. An appropriate understanding of the possible impact caused by MU could be helpful for the optimal use of tumor markers in clinical practice. In this regard, the estimation of MU and establishment of a reliable reporting range could have practical utility in the interpretation of tumor markers. As well, the establishment of MU within a narrow range is crucial in that this can contribute to increasing test reliability and ultimately reduce the proportion of test results falling outside of the reliability range as much as possible. The previous article reported a permissible

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MU limit of 15.97% for tumor markers.[16] The purpose of this study was to estimate the MU range of ovarian cancer-related tumor markers with a top-down approach, to establish their reliable reporting ranges using MU, and, in addition, to assess the proportion of test results of tumor markers not within the reliable reporting range when applied to real patients' data.

Methods

Reference intervals of tumor markers:

Tumor markers CA 125 and HE4 were measured with the Abbott Alinity i system using dedicated calibrators and reagents from Abbott (Abbott Laboratories, Abbott Park, IL, USA). The assay systems were based on chemiluminescent microparticle immunoassay.

A cut-off value is a specific threshold used to interpret laboratory test results. Test results above, below, or within a certain cut-off help categorize patients for clinical decisions such as diagnosis or risk assessment. IQC is a routine laboratory practice that uses control samples with known concentrations to monitor the consistency and reliability of analytical instruments and procedures.

Based on the package insert's date, 94.4% of healthy female subjects had CA 125 values at or below 35.0 U/mL, which was the reference interval for the CA 125 assay in the hospital where this study was conducted. As for the HE4 assay, 96 % of the healthy premenopausal subjects had an HE4 assay value at or below 70 pmol/L, and 95% of the healthy postmenopausal subjects had an HE4 assay value at or below 140 pmol/L. Based on this data, the reference intervals of the HE4 assay were at or below 70 pmol/L and at or below 140 pmol/L for premenopausal and postmenopausal female subjects, respectively. Eventually, CA 125 over 35.0 U/mL, HE4 over 70 pmol/L in premenopausal women, and HE4 over 140 pmol/L in postmenopausal women suggested a high risk of epithelial ovarian cancer.

ROMA value (%) was obtained using predictive index (PI) that was calculated with CA 125 and HE4, both in premenopausal and postmenopausal status.[13]

Premenopausal PI = -12.0 + 2.38LN[HE4] + 0.0626LN[-CA125]

Postmenopausal PI = -8.09 + 1.04LN[HE4] + 0.732LN[-CA125]

ROMA value (%) = $(e^{PI} / 1 + e^{PI}) \times 100$

The cut-off point of the ROMA value in the Abbott assays was 7.4% in premenopausal women and 25.3% in postmenopausal women. Consequently, ROMA values of 7.4% and over in premenopausal and 25.3% and over in postmenopausal women suggested a high risk of epithelial ovarian cancer, while the values of less than 7.4% and 25.3% suggested a low risk.

Estimation of MU for CA 125, HE4, and ROMA value:

IQC was performed using dedicated three-level QC material provided by the manufacturer Abbott (Alinity i; Abbott Diagnostics, Chicago, IL, USA), and IQC data were collected over one year, specifically from May 2023 to April 2024. We used a single immunoassay measurement system and two concentration levels of IQC materials (Alinity i CA125 control and Alinity i HE4 control), including multiple reagent lots during the study period. The coefficient of variation (CV) is the standard deviation expressed as a percentage of the mean.[17] And maximal CV was determined to be the standard MU of each assay. The calculation process of MU was performed as described in the previous study. That is, IQC values obtained from each reagent lot were collected separately, and standard uncertainties for each lot subgroup were combined to obtain the overall uncertainty.[18] For each assay, expanded uncertainty was determined by multiplying the standard MU by 1.65, the coverage factor, used for a one-sided 95% level of confidence (**Figure 1**).

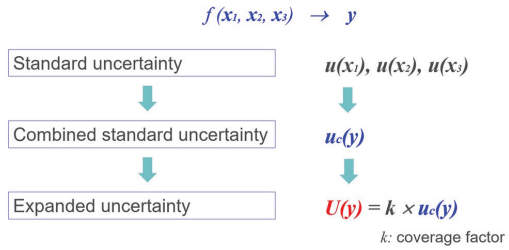

Figure 1: Overview of measurement uncertainty estimation. The scheme of measurement uncertainty from standard uncertainty to expanded uncertainty is depicted.

For the uncertainty estimation of ROMA value, standard measurement uncertainties of CA 125 and HE4 were error propagated to have combined uncertainty, and the expanded uncertainty was determined by multiplying the combined uncertainty by 1.65, the coverage factor used for a one-sided 95% level of confidence.

Calculation of the reliable reporting range for CA 125:

Reliable reporting ranges for CA 125 were assessed with the estimated expanded uncertainty of CA 125. If the measured CA 125 was smaller than or equal to 35.0 U/mL, the measured CA 125 plus expanded uncertainty should be smaller than 35.0 U/mL to be reliably at low risk. If the measured CA 125 was greater than 35.0 U/mL, the measured CA 125 minus expanded uncertainty should be greater than 35.0 U/mL to be reliably at high risk. With these calculations, a reliable reporting interval for CA 125 was determined.

Calculation of the reliable reporting range for HE4:

Reliable reporting ranges for HE4 were determined with the estimated expanded uncertainty of HE4 in premenopausal and postmenopausal women separately. For premenopausal women, if the measured HE4 was smaller than or equal to 70 pmol/L, the measured HE4 plus expanded uncertainty should be smaller than 70 pmol/L to be reliably at low risk. If the measured HE4 was greater than 70 pmol/L, the measured HE4 minus expanded uncertainty should be greater than 70 pmol/L to be reliably at high risk. For postmenopausal women, if the measured HE4 was smaller than or equal to 140 pmol/L, the measured HE4 plus expanded uncertainty should be smaller than 140 pmol/L to be reliably at low risk. If the measured HE4 was greater than 140 pmol/L, the measured

HE4 minus expanded uncertainty should be greater than 140 pmol/L to be reliably at high risk. With these calculations, a reliable reporting interval for HE4 in postmenopausal women was determined.

Calculation of the reliable reporting range for the ROMA value:

The reliable reporting ranges for the ROMA value were determined by the comparable method as HE4. For premenopausal women, the calculated ROMA value smaller than 7.4% plus expanded uncertainty should be less than 7.4 % to be reliably at low risk. While the calculated ROMA, larger than 7.4% minus expanded uncertainty, should be greater than 7.4% to be reliably at high risk. For postmenopausal women, adding expanded uncertainty to the calculated ROMA value, smaller than 25.3%, should be smaller than 25.3% to be reliably at low risk. On the other hand, extracting expanded uncertainty from the calculated ROMA value larger than 25.3% should be greater than 25.3% to be reliably at high risk.

Proportion of CA 125, HE4, and ROMA test results of real patients not within the reliable reporting range:

The tumor markers of real patients were extracted from data sources already established from September 2023 to August 2024 in the hospital where this study was conducted. As clinical laboratories are not knowledgeable about the menopausal status of each patient, HE4 and ROMA values were analyzed for both premenopausal and postmenopausal respectively.

The proportions of the test results that did not fall within the reliable reporting range were obtained by calculating the aspect of the above and below cut-off value of CA 125, HE4, and ROMA value, respectively.

Results and Discussion

Results:

Estimation of MU for CA 125, HE4, and ROMA value:

Based on the internal quality control (IQC) data for a year, standard measurement uncertainties of CA 125 and HE4 were 4.59 % and 5.21%, respectively. And expanded uncertainties of CA 125 and HE4 were 7.57% and 8.60%, respectively. Also, uncertainty estimation of the ROMA value was performed as follows. With the error propagation of standard measurement uncertainties of CA 125, 4.59%, and HE4, 5.21%, the combined uncertainty of the ROMA value was determined to be 6.94%, and the corresponding expanded uncertainty was 11.46%.

Reliable reporting range for CA 125:

The reliable reporting range for CA 125 was below 32.5 U/mL and above 37.9 U/mL. That is, CA 125 between 32.5 U/mL and 37.9 U/mL was deemed to be within the borderline range, so their risk assessment was not reliable by a single measurement (Figure 2).

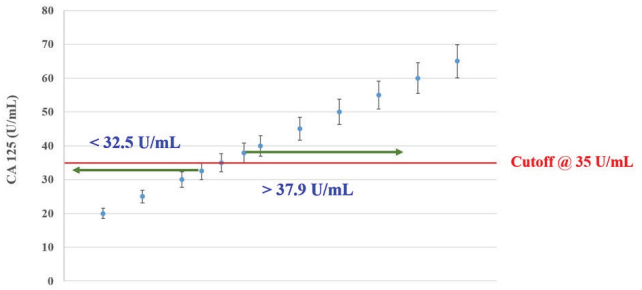

Figure 2: Measurement uncertainty-based estimation of the cutoff for CA 125. Each data point denotes representative CA 125 values such as 20, 25, 30, 35, 40, etc. U/mL with corresponding expanded uncertainties. For the CA 125 value of 32.5 U/mL, 'CA 125 + expanded uncertainty' is close to 35 U/mL, and for the CA 125 value of 37.9 U/mL, 'CA 125 – expanded uncertainty' is close to 35 U/mL. Therefore, the reliable reporting range for CA 125 is below 32.5 U/mL or above 37.9 U/mL.

Reliable reporting range for HE4:

Reliable reporting range for HE4 in premenopausal women was below 64.5 pmol/L or above 76.6 pmol/L. For postmenopausal women, the reliable reporting range of HE4 was below 128.9 pmol/L or above 153.1 pmol/L. Thus, HE4 between 64.5 pmol/L and 76.6 pmol/L in premenopausal women, as well as between 128.9 pmol/L and 153.1 pmol/L in postmenopausal women, was regarded to be within the borderline range (**Figure 3**).

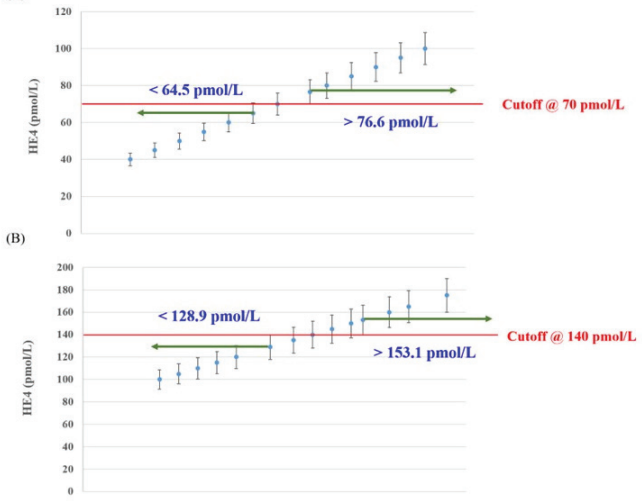

Figure 2: Measurement uncertainty-based estimation of cutoff for HE4 for (A) premenopausal and (B) postmenopausal status. (A) Each data point denotes representative HE4 values such as 40, 45, 50, 60, 70, 80, etc. pmol/L with corresponding expanded uncertainties under the assumption of premenopausal status. For the HE4 value of 64.5 pmol/L, 'HE4 + expanded uncertainty' is close to 70 pmol/L, and for the HE4 of 76.6 pmol/L, 'HE4 – expanded uncertainty' is close to 70 pmol/L. Therefore, the reliable reporting range for HE4 is below 64.5 pmol/L or above 76.6 pmol/L in premenopausal status. (B) Each data point denotes representative HE4 values such as 100, 120, 140, 150, 160, etc., pmol/L with corresponding expanded uncertainties under the assumption of postmenopausal status. For the HE4 value of 128.9 pmol/L, 'HE4 + expanded uncertainty' is close to 140 pmol/L, and for the HE4 of 153.1 pmol/L, 'HE4 – expanded uncertainty' is close to 140 pmol/L. Therefore, the reliable reporting range for HE4 is below 128.9 pmol/L or above 153.1 pmol/L in premenopausal status.

Reliable reporting range for the ROMA value:

The reliable reporting range for the ROMA value in premenopausal women was below 6.7% or above 8.4%. For

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postmenopausal women, the reliable reporting range of the ROMA value was below 22.7% or above 28.6 %. Thus, ROMA values between 6.7% and 8.4 % in premenopausal women, as well as between 22.7% and 28.6%, were considered within the borderline range (**Figure 4**).

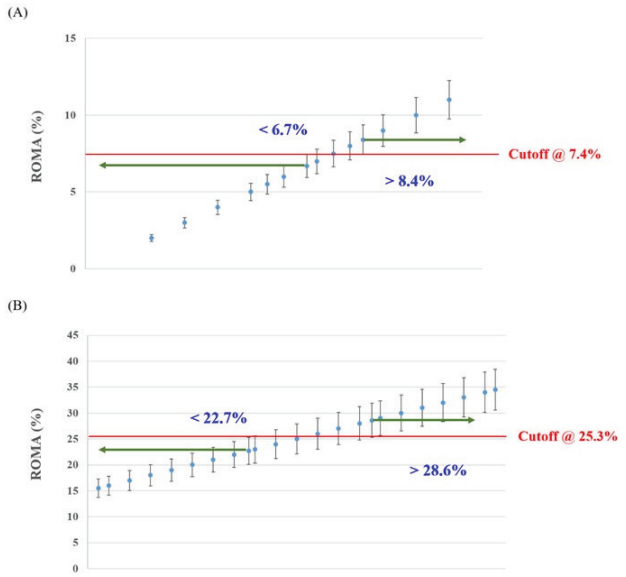

Figure 4: Measurement uncertainty-based estimation of cutoff for ROMA values for (A) premenopausal and (B) postmenopausal status. (A) Each data point denotes representative ROMA (%) values, such as 3, 4, 6, 7, 8, 9, etc. % with corresponding expanded uncertainties under the assumption of premenopausal status. For the ROMA value of 6.7%, 'ROMA + expanded uncertainty' is close to 7.4%, and for the ROMA of 8.4%, 'ROMA – expanded uncertainty' is close to 7.4%. Therefore, the reliable reporting range for ROMA is below 6.7% or above 8.4% in premenopausal status. (B) Each data point denotes representative ROMA (%) values such as 20, 22, 25, 28, 3,0, etc. % with corresponding expanded uncertainties under the assumption of postmenopausal status. For the ROMA value of 22.7%, 'ROMA + expanded uncertainty' is close to 25.3%, and for the ROMA of 28.6%, 'ROMA – expanded uncertainty' is close to 25.3%. Therefore, the reliable reporting range for ROMA is below 22.7% or above 28.6% in premenopausal status.

Reliable reporting range for CA 125 in patients' data:

3,501 CA 125 tests, consisting of 494 CA 125 tests independently performed and 3,007 ROMA value tests, were included. Among 3,501 CA 125 results, 2,195 results were below or equal to 35.0 U/mL, which was the cut-off value, and 1,306 results were above 35.0 U/mL. Among the 2,195 results, 2,121 results were below 32.5 U/mL, and therefore 74 results didn't belong to the reliable reporting range. Also, among the 1,306 results, 1,212 results were above 37.9 U/mL, which meant that 94 results were not included in the reliable reporting range. To sum up, 168 out of 3,501 (4.8%) test results could be regarded as those belonging to the borderline range.

Reliable reporting range for HE4 in patients' data:

Finally, 5,493 HE4 test results, composed of independent 2,486 HE4 tests and 3,007 ROMA value tests, were collected. Assuming that all the tests were obtained from premenopausal women, the cut-off value was determined as 70 pmol/L. Among the 4,414 results below or equal to 70 pmol/L, 4,285 results estimated below 64.5 pmol/L lay in the reliable reporting range, except 129 results out of this range. On the other hand, among the 1,079 results above 70 pmol/L, 952 results measured above 76.6 pmol/L were included in the reliable reporting range, while 127 results were not. Ultimately, 256 out of 5,493 (4.7%) test results could be regarded as those belonging to the borderline range.

Meanwhile, if all of them were obtained from postmenopausal female subjects, a 140 pmol/L cutoff value was applied. Of the 5,046 results below or equal to 140 pmol/L, 5,005 results below 128.9 pmol/L were within of reliable reporting range, and eventually, 41 results did not belong to this range. Also, among the 447 results above 140 pmol/L, 407 results above 153.1 pmol/L were included in the reliable reporting range, but 40 results were not. At last, 81 out of 5,493 (1.5%) test results were regarded as those belonging to the borderline range.

Reliable reporting range for the ROMA value in patients' data:

With the assumption of premenopausal status, the cut-off for high risk was defined as a ROMA value was 7.4% or more. A 2,204 of 3,007 test results were below 7.4%, and 803 were above or equal to 7.4%. Among the 2,204 results, 2,117 results below 6.7% were included in the reliable reporting range, while the other 87 results were not. As well, among the 803 results, 689 results above 8.4% fell within the reliable reporting range, and 114 results didn't. To sum up, 201 out of 3,007 (6.7%) test results were regarded as those belonging to the borderline range. With postmenopausal status assumed, 25.3% instead of 7.4% was applied as the cut-off value. Among the 2,328 results below 25.3%, aside from 2,244 results estimated below 22.7%, 84 results didn't belong to the reliable reporting range. Also, among the 679 results above or equal to 25.3%, 580 results above 28.6% were included in and 99 results were not included in the reliable reporting range. Consequently, 183 out of 3,007 (6.1%) test results were regarded as those belonging to the borderline range (**Figure 5**).

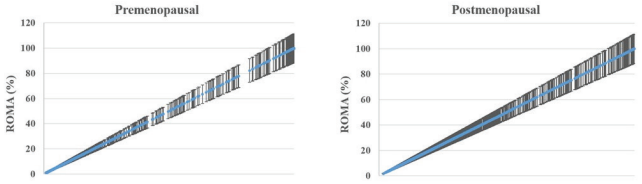

Figure 5: ROMA values in real patients' data with expanded uncertainties expressed using the formulae for premenopausal and postmenopausal status. Even for the same data pair of CA 125 and HE4, the ROMA values are different depending on the menopausal status. These differences are reflected in two figures.

Table 1: Summary of results.

Tumor Marker / Parameter	Standard Measurement Uncertainty (CV, %)	Expanded Uncertainty (%) (k=1.65)	Reliable Reporting Range
CA 125	4.59	7.57	<32.5 U/mL or >37.9 U/mL
HE4 (Premenopausal)	5.21	8.60	<64.5 pmol/L or >76.6 pmol/L
HE4 (Postmenopausal)	5.21	8.60	<128.9 pmol/L or >153.1 pmol/L
ROMA (Premenopausal)	6.94	11.46	<6.7% or >8.4%
ROMA (Postmenopausal)	6.94	11.46	<22.7% or >28.6%

Discussion:

According to this study, expanded MU of ovarian cancer related tumor markers were measured about 7.6-11.5% and about 4-6% of real patients' tumor markers were not included in reliable reporting range, which meant the assessment for ovarian cancer could not be decisively made by tumor markers level in the approximately 4-6% of all tests obtained in our laboratory.

Biomarkers, including tumor markers, play a role as risk factors and predictors of clinical outcomes and are usually presented as continuous types, which are often divided into two categories based on the cutoff point. Although this categorization provides physicians with distinctively presented criteria, the existence of a borderline around the cutoff value should be cautiously considered to avoid an unintended wrong decision. In a clinical practice setting, the test results placed near to cut-off points are not enough convincing to lead to a confirmatory decision.[19]

As well, although the cut-off value is decided in a way that test results of 95% among healthy subjects are included within the range bordered by this value, the intrinsic uncertainty of test results eventually alleviates its absolute significance. In this regard, a reliable reporting range is needed and determined using the MU obtained by CV. On the other hand, the area outside of the reliable range is defined as the borderline range, located close to the cut-off value. Test results within the borderline range enhance the necessity of retesting markers or referring to other laboratory or radiological results combined with clinical assessment.[2]

Especially because tumor markers act as the indicator of diagnosis, disease severity, progression, and treatment effects, misinterpretations of tumor markers can cause not only unwanted results such as misdiagnosis, unnecessary treatment, and economic burden, even serious or fatal consequences in comparison with other disease entities.[20,21] The application of MU in this field is supposed to be more crucial than other disease entities.

MU is an important indicator reflecting the performance characteristics of clinical testing, but its recent introduction into clinical laboratories has rendered the laboratory use limited.[1] Estimated uncertainties are often accompanied by measured reference values in reference laboratories, but they are rarely reported in routine clinical laboratory testing. This study applied MU to clinical use.

The top-down and bottom-up approaches were used for estimating MU. The study comparing the MU between the two approaches indicated that the MU obtained by the bottom-up approach was quite similar to that obtained by the top-down approach.[22] This result supports the top-down approach, used in this study, more than the bottom-up approach taken. The top-down approach is simpler and more practical in routine laboratory settings. The top-down approach is now officially endorsed by the ISO 20914, which provides practical guidance to be applied in medical laboratory settings for the purpose of estimating MU of values produced by measurement procedures intended to measure biological measurands.[23]

It estimates the MU of laboratory results by using IQC data to derive the random components of uncertainty and commercial calibrator information. It is based on the premise that long-term IQC results sufficiently reflect error factors that can change the test value, and is known to be useful in modern clinical laboratories, especially when closed measurement systems are used.[2] Therefore, we collected IQC results of CA 125 and HE4 for one year and determined the standard measurement uncertainties of CA 125 and HE4 based on CVs.

In general, when the 95% confidence interval is assumed, combined uncertainty is multiplied by the coverage factor 2 to calculate expanded uncertainty. But in case of the clinical tests used for comparison with reference interval or clinical decision limit, as in this study, a one-sided 95% level of confidence is assumed, so k=1.65 is multiplied by the combined uncertainty to obtain expanded uncertainty. As for the uncertainty estimation of the ROMA value, the combined uncertainty is calculated by the error propagation of the standard measurement uncertainties of CA 125 and HE4.[2]

Due to the different distribution of HE4 by menopausal status, the reference interval of HE4 is determined differently according to menopausal status. As uncertainty is expressed as CV percent, the bigger the test results, the bigger the uncertainty, even for the same test item. Therefore, values bigger than cutoffs have bigger uncertainty compared with those smaller than cutoffs, and, therefore, HE4 results in postmenopausal status have a broader borderline range compared with those in premenopausal status.

ROMA value is predominantly influenced by HE4 results in premenopausal status and by both CA 125 and HE4 in postmenopausal status.[24] Generally, higher CA 125 and HE4 results are obtained in postmenopausal women, and thus higher ROMA values are calculated in postmenopausal females with a broader range of borderline values. The interpretation of results far from cutoffs is generally unequivocal, but results close to cutoffs should be interpreted with caution, and the reliable reporting range should be considered as well as the measured values themselves. As MU is mostly determined by the precision parameter, CV performance characteristics, such as the imprecision of the testing method, are of critical importance for the reliability of test results. That is the reason why a more precise testing method should be utilized in clinical laboratory practices.

The current study's results indicated that about 4-6% of tumor markers of ovarian cancer were included in the borderline range. Considering that this range can be variable among laboratories depending on the assay systems or selected patients' characteristics, even values within the reliable reporting range should be prudently interpreted along with a comprehensive understanding of clinical aspects and other laboratory data.[25]

Applying MU estimation in clinical laboratories remains challenging due to the absence of standardized formulas and universally accepted limits for MU calculation and interpretation. Furthermore, communication of MU to clinicians is often inadequate, diminishing its clinical utility. Future efforts should focus on developing standardized, practical protocols for MU estimation, reporting, and interpretation, alongside

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educational initiatives to enhance awareness and effective use of MU data in clinical decision-making.

This study has several limitations. First, MU estimates were derived solely from IQC data on a single analytical platform, which may restrict the applicability of findings to other assay systems. Second, the MU estimation did not incorporate external quality assessment data or biological variation, both of which could provide complementary perspectives. Third, the inherent variability of tumor marker assays, combined with the absence of internationally standardized reference materials for ovarian cancer markers, might influence the comparability of results. Fourth, this was a single-center study, so the generalizability of the findings might be limited. Further multicenter studies would be warranted to validate and extend these results.

Conclusion

In this study, we assessed the statistical characteristics of ovarian cancer-related tumor markers using MU principles and determined a possible reliable reporting range for them. Also, the distribution of results belonging to the borderline range was assessed using real-world patients' data. The findings shown in this study could be a guide to the interpretation of test results in clinical practices, which could further help physicians to make diagnoses, establish treatment methods, and evaluate treatment outcomes. In the future, more precise MU would enhance the clinical value of tumor markers as accurate assessment tools.

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