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Editorial

Lung cancer tumor marker analysis: A clinical laboratory perspective

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Abstract. Clinical laboratories are responsible for performing lung cancer tumor marker testing as part of routine clinical care. It is their responsibility to guarantee that the reported tumor marker results are reliable and meet the necessary quality standards for proper clinical use. During the different laboratory phases, pre-analytical, analytical and post-analytical, specific steps and processes can introduce errors and generate incorrect clinical interpretation. This editorial briefly outlines critical laboratory issues related to lung cancer tumor markers, specific for each of these three laboratory phases.

Keywords: Tumor markers, clinical laboratory, clinical chemistry, lung cancer

1. Introduction

In contrast to research laboratories, test results generated by clinical laboratories are directly used in patient care, and therefore require a high quality standard, demanding accuracy and reliability. There are plenty of national and international regulations for the use of tumor marker assays in patient care. In the European Union, the new *in vitro* diagnostic medical devices regulation (IVDR) requires extensive assay validation and comprehensive documentation of both analytical and clinical performance by the manufacturer or the applying lab, in order to guarantee a high level of certainty regarding the accuracy of measurement and interpretation of tumor marker results. Most clinical laboratories must comply with local legislative requirements and generally have an operational quality management system in place, most often based on the ISO15189 norm [1]. Furthermore, they generally undergo external auditing and accreditation. These systems are not specific for tumor markers, but include all operational clinical tests. Many aspects of the quality management systems relate to organizational and personnel requirements and having systems in place to detect and prevent errors. Improvement cycles are utilized to prevent errors to recur in the future. On a more operational level, laboratory errors can occur in the pre-analytical, analytical, and post-analytical laboratory processes. While many issues are not unique to (lung cancer) tumor markers, some specific issues can be identified that could potentially compromise the quality and reliability of the results. To address these issues, an initiative has recently been launched to provide guidance and recommendations to clinical laboratories. This effort involves the development of a Clinical & Laboratory Standards Institute (CLSI) guideline for

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biochemical tumor marker analysis (CLSI C65), for the most widely used tumor markers. It is expected that this document becomes available in 2024. For now, this editorial outlines some specific issues associated with enabling accurate and reliable results for tumor markers available for lung cancer care.

2. Pre-analytical phase

The pre-analytical phase encompasses the clinical laboratory processes preceding the actual measurement of a tumor marker in the obtained sample. This includes the test ordering, blood collection procedure for a specific clinical question, the preparation of the patient, performing the blood collection procedure, sample transportation, blood-sample processing and storage/stability prior to analysis. It has to be pointed out that of all laboratory errors that may occur, it is generally acknowledged that more than 70% are related to the pre-analytical phase.

First of all, the test ordering and the tumor marker selection must be relevant to the individual patient characteristics. For instance, certain lung cancer tumor markers, like neuron-specific enolase (NSE) and progastrin-related peptide (ProGRP), might be more relevant for follow-up of small cell lung cancer (SCLC), while others, such as carcinoembryonic antigen (CEA), cytokeratin-19 fragments (CYFRA 21-1) and squamous cancer cell antigen (SCCA), are more relevant for non-small cell lung cancer (NSCLC) [2, 3]. In addition, the clinical setting is crucial, as most tumor markers currently have limited value for lung cancer screening but can be highly relevant for monitoring of systemic treatment in advanced lung cancer [4]. Also the timing of tumor marker testing is important and must be aligned with key clinical decision moments and provide relevant additional clinical information for the specific clinical context. This may vary depending on treatment. For example, daily tumor marker measurement in lung cancer patients is unlikely to yield informative results but would incur additional costs.

When reviewing the blood collection procedure, several potential risks not specific to tumor markers, such as patient and sample mix-up, have to be managed. For tumor markers, the choice of blood collection tube and matrix can be crucial and should therefore be validated for suitability. For instance, NSE cannot be analyzed in plasma obtained from an anti-coagulated blood collection tubes, while for other tumor markers, this consideration may be less important. Another pertinent issue that can significantly compromise the accuracy of tumor marker results is the occurrence of *in vitro* hemolysis. Two tumor markers, NSE and lactate dehydrogenase (LDH), are among the analytes most sensitive to *in vitro* hemolysis. Even minimal erythrocyte lysis can falsely increase the concentrations of these tumor markers, as they are released from the erythrocytes [5, 6]. The other pre-analytical processes that potentially affect the accuracy and reliability of lung cancer tumor markers are sample management, transport, and storage. In this edition of Tumor Biology, Canki et al. have validated the most relevant of these processes for the CEA, CYFRA 21-1, CEA, CA125, NSE, and HE4 tumor markers. Specifically, they assessed whole blood stability, freeze-thaw cycle stability, electric vibration mixing stability, and storage stability of obtained serum at refrigerated (4°C) and room temperatures (20°C) [7]. They described pre-analytical restrictions that have to be taken into account to ensure accurate and reliable test results for these tumor markers. Each clinical laboratory must validate the suitability of the relevant pre-analytical variables for each tumor marker, either directly (themselves) or indirectly (by vendor or other clinical laboratory). With this information, the pre-analytical requirements for accurate and reliable tumor marker reporting have to be assured by implementing the relevant standard operating procedures.

3. Analytical phase

The analytical phase involves the actual measurement of the tumor marker using a specified measurement procedure. In general, protein-based tumor markers, such as CEA, CYFRA 21-1, NSE, ProGRP,

SCC, CA125, etc., are measured using sandwich or immunometric immunoassay designs, mainly on automated instruments. For most tumor markers the measurement procedures from different vendors use different assay designs in terms of antibody epitope recognition and labels used to readout the immunoassay signal. These differences can affect the immunoassay performance. This heterogeneity of immunoassay designs together with the clinical occurrence of tumor marker isoforms can limit the comparability of the different tumor marker tests for individual clinical samples [8]. Another issue associated with some tumor markers, including CEA and CA125, is the known lack of harmonization status for the measurement systems used in today's clinical practice or even uncertainty regarding the current harmonization status [9, 10]. This is caused, among other factors, by a lack of commutable international standards for these tests. Altogether, these issues complicate the comparability between measurement procedures.

Therefore, when transitioning to a new measurement procedure in a clinical lab, a thorough method comparison is required to confirm comparability. Sometimes, simultaneous measurement using both procedures is required for clinical samples, to prevent clinically significant differences for individual patients and avoid clinical misinterpretation. It has to be pointed out that, for these reasons, most often, serial values can only be reliably interpreted if the same method (manufacturer, analyzer) is used.

4. Post-analytical phase

The post-analytical phase includes the transfer of a measurement result to the final medical record and clinical interpretation thereof. In general, the transfer to the final report, whether digitally or on paper, is highly automated and not specific for tumor markers. The reporting of tumor markers is generally supported by either reference ranges or sometimes clinical decision limits. The reference ranges are generally defined by the 95% interval of results observed in a population of apparently healthy volunteers. These ranges can be specified for gender, such as for CA125, or age categories (or menopausal status), as seen with HE4, and affected by other (environmental) factors, such as smoking for CEA. Since tumor markers are generally only relevant when elevated, the cut-off can be based on either the one-sided or two-sided 95% interval. When clinical decision limits are reported, these are based on either diagnostic or clinical relevance and utility. Furthermore, clinical interpretation of tumor markers also requires consideration of other benign causes of (falsely) elevated tumor markers, such as renal failure, hepatitis, etc. A highly relevant overview of elevated tumor markers resulting from benign conditions has been provided and updated by Trape et al. in this special edition of Tumor Biology [11].

Since most tumor markers for lung cancer are not used in a diagnostic setting, but primarily during follow-up of patients, the interpretation of tumor markers is generally based on changes in concentration over time. The so-called reference changed value (RCV) has been suggested for this purpose [12, 13]. However, some limitations of the RCV have been acknowledged, and therefore it should be used with caution when interpreting longitudinal tumor marker results [14]. One major point of criticism is that RCVs are derived from healthy individuals, while cancer patients – particularly those in advanced stages – often exhibit highly elevated tumor marker levels with potentially different variation profiles. Therefore, new methodologies for interpreting the longitudinal tumor markers are necessary. Some examples of work on this topic include the development of so-called biomarker response characteristic (BReC) plots [15] and advanced modeling of longitudinal data to enable accurate prediction of relevant clinical events in lung cancer patients [16]. Such strategies have proven to provide relevant diagnostic performance in advanced lung cancer [17, 18]. This Tumor Biology edition includes a multi-longitudinal study by van Delft et al. which takes a further step and incorporates longitudinal data of multiple lung cancer tumor markers [19]. These approaches show that individual tumor marker

kinetics are much more relevant than absolute thresholds in patients with advanced cancer stages – and the reference range becomes less relevant. All the more, the rate of decrease or increase that is clinically meaningful has to be defined for each marker and each type of cancer. This defines also the time intervals when the markers are investigated. Obviously, the biological variability, pre-analytical influences and analytical variation have to be taken into account for the interpretation of the kinetics, which may vary for each analyte and method. Thus, further developments in terms of methodologies, as well as the practical application of developed methods and models, are warranted.

5. Technical quality assurance and control

A final topic worth discussing is the technical quality assurance and control for tumor markers. This includes the operational systems clinical laboratories have in place to prevent or detect errors. Clinical laboratories have several tools at their disposal for this purpose, including external quality control, internal quality control, auto-verification, and clinical validation of results [20]. External quality control is performed by participating in an external quality control program (EQA) that sends-out samples to multiple laboratories for analysis. This enables comparison with peers and, particularly for tumor markers, with other laboratories using the same or highly similar measurement procedures. Most EQA tumor marker programs use artificial samples, which may behave differently than true patient samples, and therefore may not be suitable for comparing measurement procedures from different vendors. Internal quality control includes measuring quality control samples to confirm appropriateness of the measurement system on a more daily basis and is used to regularly check the quality of the measurement system [20]. Internal quality control also serves to prevent errors by checking the quality of a measurement system after procedures associated with a risk of error, such as calibration or analyzer maintenance, prior to analyzing clinical samples. Another tool available for laboratories are checks based on obtained patient results which can trigger follow-up actions [20]. These checks can be based either on the value itself (limit check), e.g. a very high tumor marker result, the change in value (delta check), or any specific combination(s) of results (multivariate check). These checks are used to enable detection of pre-analytical, analytical, or post-analytical errors, and provide clinical consultation by the laboratory specialist or clinical pathologist. Some recent studies have investigated such checks for tumor markers. However, setting-up these criteria remains a challenging task and is rather laboratory-specific, particularly depending on factors such as the patient populations, operational risks associated with local tumor marker analysis, and the clinical impact of tumor marker test results.

6. Conclusion

Clinical laboratories that offer lung cancer tumor markers for the routine clinical practice have to ensure that reported test results are reliable and meet specified quality standards. Therefore, clinical laboratories typically have a quality system in place which is controlled by external accreditation agencies. Potential errors can originate from the pre-analytical, analytical and post-analytical phases of laboratory testing, each of which has its specific issues and risks for errors. To manage these potential risks, clinical laboratories have several tools at their disposal to ensure the reporting of accurate and reliable tumor marker test results.

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Author contributions

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Revision for important intellectual content: SH, HvR Supervision: SH, HvR.

Conflict of interest

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