Review

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Clinical usefulness of circulating tumor markers

https://doi.org/10.1515/cclm-2022-1090
Received October 28, 2022; accepted November 2, 2022;
published online November 17, 2022

Abstract: Tumor markers are a heterogeneous group of substances released by cancer cells into bloodstream, but also expressed by healthy tissues. Thus, very small concentrations can be present in plasma and serum from healthy subjects. Cancer patients tend to show increased levels correlating with tumor bulk, but false positive results could be present in patients with benign conditions. The correct interpretation of TM results could be challenging and many factors should be considered, from pre-analytical conditions to patient concomitant diseases. In this line, the Clinical Chemistry and Laboratory Medicine journal has made important contributions though several publications promoting the adequate use of TM and therefore improving patient safety. TM measurement offers valuable information for cancer patient management in different clinical contexts, such as helping diagnosis, estimating prognosis, facilitating early detection of relapse and monitoring therapy response. Our review analyzes the clinical usefulness of tumor markers applied in most frequent epithelial tumors, based on recent evidence and guidelines.

Keywords: cancer detection; follow up; tumor markers.

Introduction

Classically, the term tumor marker (TM) refers to substances directly produced by cancer cells or by other cells in response to a tumor. Circulating TMs are present in the blood, and other body fluids, like urine and pleural or peritoneal effusions. Table 1 summarizes the main TMs used in clinical practice, showing the associated malignancies. The clinical role of circulating TMs has remained invariable over the years and their usefulness controversial, in spite of their wide potential application in cancer diagnosis, prognosis and monitoring (Table 2).

TMs are cancer related substances but they are not specific, as they can be also expressed by healthy tissues, and small concentrations could be detected in healthy subject's bloodstream. Cancer patients may have raised levels of TMs, which are correlated with the disease stage. However, false positive results could be found in patients with benign conditions, including processes that increase their release or reduce their catabolism. Increased plasma concentrations of TMs could lead to unnecessary tests to confirm or rule out the suspected neoplasm and also psychological impact on patients. In order to avoid these undesirable effects, it is essential to know the potential causes of false positives. On this point, the Clinical Chemistry and Laboratory Medicine (CCLM) journal, through several publications, has facilitate the knowledge on physiopathological processes that increase the concentrations of TMs [1].

This review analyzes the usefulness of most used TMs in frequent epithelial tumors, based on recent evidence and clinical guidelines.

The role of tumor markers according to clinical guidelines

In recent years, there has been a notable increase in requests for circulating TMs, mostly due to inadequate use of their measurement [2]. In a large study published in CCLM, Moreno-Campoy et al. [3] indicate that only 39.88% requests out of 23,059 in 5,080 patients with neoplastic diseases have been classified as adequate according to current clinical guidelines. In fact, assessing the adequacy of TMs according to their clinical usefulness remains the main challenge.

Based on available scientific evidence, clinical practice guidelines should be used to define, in which cases TMs are clinically valid and, therefore, offer an improvement in clinical results and the patient's quality of life, also contributing to maintain an adequate financial balance.

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Table 1: Main tumor markers used in clinical practice.

Tumor marker	Associated malignancies
Alpha-fetoprotein (AFP)	Hepatocellular carcinoma, germ cell tumor
Beta-2-microglobulin	Multiple myeloma
Beta chorionic gonadotropin	Choriocarcinoma, germ cell tumor
CA 125	Ovarian, lung, endometrial
CA 15-3	Breast
CA 19-9	Pancreas, biliary tract, colorectal, gastric, ovarian (mucinous tumor)
CA 72-4	Stomach, ovarian
Calcitonin	Thyroid (medullary)
Carcinoembryonic antigen (CEA)	Colorectal, gastric, esophageal adenocarcinoma, non small cell lung cancer, breast
Chromogranin A	Neuroendocrine tumors
CYFRA 21-1	Non-small-cell lung cancer
Her-2-neu	Breast
HE-4	Ovarian
Neuron specific enolase (NSE)	Neuroendocrine tumors, small cell lung cancer
ProGRP	Snall cell lung cancer
Total and free prostate specific antigen (PSA)	Prostate
S100	Malignant melanoma
Squamous cell carcinoma antigen (SCC)	Squamous cancers
Thyroglobulin	Thyroid

Table 2: Potential clinical utility of circulating tumor markers.

Clinical utility
Assessment of cancer risk
Screening in asymptomatic population
Early diagnosis of cancer
Prognosis and selection of treatment
Assessment of radicality in patients treated with curative surgery or radiotherapy
Early detection of relapse
Monitoring therapy

However, clinical guidelines do not always agree and, occasionally their recommendations can be controversial. The extensive tumor-by-tumor review on available guidelines carried out by Gion et al. [4–6], represents a valuable contribution. With the Appraisal of Guidelines for Research & Evaluation (AGREE II) tool, this review provides assessment of each guide to facilitate quality comparison.

These guidelines, despite their overall quality rating is high, do not always consider sufficiently the pre-analytical, analytical and post-analytical variables that are involved in the measurement of TMs. Among the guidelines that do take this into account is the National Academy of Clinical Biochemistry (NACB), which, despite not having been updated since 2008, presents a broad description of the laboratory variables that can affect the TMs measurement [7, 8]. All the variables that affect the analytical process, from pre-analytical conditions to methodological differences, have a great impact on the results and their correct interpretation. Not taking these variables into account can cause significant errors in the clinical allocation of TMs. This topic has been extensively reviewed by the CCLM journal, as reflected in the following publications.

Filella et al. [9] remarked that the quality of prostate cancer (PCa) early detection guidelines could be improved properly considering the laboratory issues in their development and proposed a list of questions that should be considered regarding PSA measurement. The authors highlight the lack of interchangeability between the different PSA assays, which remains unsolved despite the introduction of international WHO standards [10, 11]. This is an aspect that needs to be emphasized for all TMs and that sometimes, as happens with CA 19-9 [12], leads to notable inter-assay differences that can cause errors in their interpretation of results when they are not considered.

Additionally, among laboratory variables not generally considered in clinical guidelines, there are differences in measured TMs concentrations attributable to the use of different reagent lots overtime. These differences can potentially cause result misinterpretation. To minimize this risk, Solsvik et al. [13] recently proposed a simplified and pragmatic lot-to-lot evaluation processing in which information about multiple lot changes from different clinical laboratories can be accumulated nationally (Table 3).

Clinical value of tumor markers in cancer detection

Diagnostic confirmation and histological classification of cancer may be challenging. Delay in the diagnostic process compromise patient's therapeutic options and consequently their prognosis. The ideal clinical usefulness of TMs is to facilitate, in a non-invasive, simple and fast way, cancer early diagnosis. However, most TMs lack sensitivity to detect tumors in early stages and therefore guidelines do not recommend them as a screening tool in asymptomatic patients. As a maximum, in patients with suggestive signs or symptoms of malignancy, the detection of elevated

Table 3: Value of PSA to predict the future diagnosis of prostate cancer.

Authors	Number of subjects included in the study	Number of prostate cancer patients	Age of baseline PSA	Results
Stenmann et al. [14]	21,172	44	45–84	PSA higher than 2.5 μg/L predicts the appearance of prostate cancer in the decade following the PSA measurement
Gann et al. [15]	22,071	366	40–84	A single PSA measurement had a relatively high sensitivity and specificity for detection of prostate cancers that arose within 4 years.
Loeb et al. [16]	13,943	661	40–59	A baseline PSA value between the age-specific median and 2.5 ng/mL was a significant predictor of later
Lilja et al. [17]	21,277	462	44–50	A single PSA test at age 44–50 years predicts subsequent clinically diagnosed prostate cancer.
Vickers et al. [18]	1,167	126	60	PSA measurement at age 60 predicts lifetime risk of metastasis and death from prostate cancer.
Preston et al. [19]	22,071	234	45–59	PSA levels in midlife strongly predict future lethal prostate cancer
Kovac et al. [20]	10,968	970	55–60	Baseline PSA levels among men aged 55–60 years were associated with long-term risk of clinically significant prostate cancer

levels would support clinical suspicion. With few exceptions, such as α fetoprotein and human chorionic gonadotrophin in germ cell tumors [21] and prostate specific antigen (PSA) in PCa, most recommendations are against their use for cancer detection. Furthermore, TMs quantification may be useful in patients with cancer of unknown primary site to suggest the origin, reducing the hospitalization time, morbidity, and the number of tests for diagnosis [22].

The role of PSA has been relevant in PCa detection, although its use has long been highly controversial [23]. PSA low specificity, the risk of overdiagnosis, overtreatment on screening, along with treatment adverse effects and a questionable decrease in mortality caused by PCa are reasons against the use of PSA on screening [24].

A recent initiative promoted by the European Association of Urology (EAU) proposes to overcome the disjunctive between screening everyone and not screening anyone, choosing to use PSA in a more effective way [25–27]. This new strategy is based on the PSA ability for predict future diagnoses of PCa shown in different studies [14–20] (Table 2). A recent study published by Kovac et al. [20], analyzing the data from 10,968 individuals enrolled in the American PLCO screening trial, remarked the strong relationship between basal PSA concentration among patients aged 55–60 and the long-term diagnosis of clinically significant PCa.

EAU initiative considers that a personalized strategy can reduce the harms effects of screening, maintaining the reduction of metastases and death, according the European Randomized Study of Screening for Prostate Cancer [28]. The algorithm proposed by EAU is applied to well-informed subjects and starts with a baseline PSA that will determine the periodicity of subsequent measurements. For subjects with a baseline PSA lower than 1 μg/L, PSA should be measured after 5 years in individuals aged 50–59, whereas PSA measurements should be stopped for subjects aged 60–70. On the other hand, PSA measurement should be done after 2–4 years when the baseline PSA is between 1 and 3 μg/L. Finally, risk stratification based on risk calculators and magnetic resonance imaging to select men for prostate biopsy is proposed when PSA is higher than 3 μg/L. Recently, the European Commission recommended the implementation of a PCa screening program based on PSA testing for men up to 70, in combination with additional magnetic resonance imaging as a follow-up test [29, 30].

One example of recommendations against the use of TM in the diagnosis setting is breast cancer (BC), the neoplasm with the highest incidence and mortality in women. In this case, a large number of circulating biomarkers have been studied, including mucins (carbohydrate antigen 15.3 [CA 15.3], cancer antigen 27–29 [CA 27–29]), oncoproteins (serum HER2 [sHER2]), oncofoetal proteins (carcinoembryonic antigen [CEA]), and cytokeratins (tissue polypeptide antigen [TPA], tissue polypeptide specific antigen [TPS] and cytokeratin-19 fragment [CYFRA 21-1]) [31]. Among all biomarkers, CA 15.3 is the most valuable; however, its sensitivity is conditioned by the cancer stage. In a prospective study including 2,062 patients with untreated

primary BC, the overall sensitivity of CA 15.3 was higher than CEA (19.6 vs. 12.7%); and the combined assessment, increased sensitivity to 28% [32]. In spite of less evidence on the use of CEA, the combination of both markers provides complementary information and increases performance [33, 34]. However, due to low sensitivity, their measurement is not recommended for screening or early diagnosis, as reflected unanimously in international clinical guidelines [34–37]. Given their relationship with disease extension, it is reasonable to think that they can be useful complementing patient staging [38]. Nevertheless, the European Group on Tumor Markers (EGTM) is the only one that supports its value for prognosis and staging, as high levels of both TMs in patients with localized disease might facilitate the detection of subclinical metastases [33].

Regarding lung cancer (LC), specific biomarkers have not been yet identified and current TMs usefulness is still unclear. Despite improvements in diagnosis approaches and novel treatments (targeted therapies and immunotherapy), LC remains the leading cause of cancer mortality worldwide. Most patients do not exhibit specific symptoms on diagnosis and one in two patients is diagnosed with advanced or locally advanced stages, thus there is an urgent need to improve tools for early diagnosis. Current strategies for LC screening are based on low-dose computed tomography (LDCT), which is supported by positive results from The National Lung Cancer Screening Trial (NLST) and the European NELSON trial [39, 40]. However, LC screening is still far from global implementation and there are concerns regarding false positives, the risk of overdiagnosis, and differences in patient selection criteria.

Circulating biomarkers would optimize imaging screening in two ways: (1) as a pre-test to refine risk stratification in combination with current selection criteria (age and tobacco exposure), and (2) as a post-test to help clinical decision-making in the management of indeterminate lung nodules (ILNs), also reducing unnecessary LDCT follow-ups [41]. Different panels of biomarkers have been evaluated but to date, none has shown sufficient sensitivity and specificity to be implemented for screening purposes. As an example, a panel of three TMs (CEA, cancer antigen 125 [CA 125], CYFRA 21-1) and 1 autoantibody (AAb) showed 71% sensitivity and 88% specificity in a selected high-risk cohort (based on age and smoking history as risk factors) [42]. However clinical validation in an independent cohort demonstrated lower sensitivity (49%) [43]. A recent study, analyzed a multi-analyte blood test to detect several cancers at early-stages. This test includes the TMs CA 125, CEA and CA 19-9; among other proteins and mutations in cell-free DNA (cfDNA). Although combining conventional TMs with novel circulating biomarkers seems a promising strategy for other cancers, the sensitivity obtained for LC was the lowest (39%) [44]. In conclusion, neither existing circulating biomarkers nor a combination of them is recommended in asymptomatic populations or specific high-risk groups.

Interestingly, circulating TMs have potential for helping diagnosis in patients with suggestive symptoms of LC (ILN, hemoptysis, dyspnea, etc.). According to the NACB, the most useful TMs aiding LC detection are: CEA, CYFRA 21-1, neuron specific enolase (NSE), squamous cell carcinoma antigen (SCC), and pro-gastrin releasing peptide (ProGRP) [45].

Additionally, the detection of ILN is very common; approximately 1.5 million nodules every year in the United States. Although the majority are benign, the effective detection and treatment of malignant nodules are crucial to reduce mortality [39]. The prospective study including 3,144 patients with clinical suspicion of LC (33% with nodules), evaluates the performance of six combined TMs obtaining an 88.5% sensitivity and 82% specificity. The inclusion of TMs results increased the detection capacity (AUC from 0.85 to 0.93) of the conventional prediction model based on nodule size, age, and smoking status [46]. Thus, the most effective strategy to improve diagnostic performance is combining multiple biomarkers with image and clinical parameters.

TMs can also aid LC diagnosis in the histological classification of tumors, as the therapeutic conduct and prognosis differ depending on it. Different patterns can discriminate between non small cell lung carcinomas (NSCLC) and small cell lung carcinomas (SCLC), also suggesting the most probable histology: increased CEA in adenocarcinoma; CYFRA 21-1 and SCC in squamous cell carcinoma; and NSE and ProGRP in small cell lung cancer [45]. Despite evidence, no clinical guideline recommends the use of TMs as a tool for diagnostic or histological discrimination. Initial evaluation of patients with suspected LC includes general laboratory tests such as hematology, coagulation, and biochemical profile, without specifying the possibility of using TMs [47]. Only the NACB and the EGTM pointed out that TMs have considerable potential for differential diagnosis and histological subtyping, particularly in tumors of unknown origin [45, 48]. The British Thoracic Society guidelines for the investigation and management of pulmonary nodules reported that although some biomarkers show interesting results, further studies are required to validate their performance prospectively prior to be recommended for clinical practice [49].

The implementation of new circulating biomarkers for early diagnosis of epithelial ovarian cancer (EOC) is an

unmet medical need. This cancer is considered as "silent killer" because it is the most deathly gynecologic malignancy, frequently diagnosed in advanced stages. Currently, CA 125 is the predominant TM in EOC, although it is not exempt from limitations, including low sensitivity and specificity. Despite the enormous efforts to discover new biomarkers, the only one that has reached an effective clinical implementation is HE4, authorized by the FDA in 2008 [50]. Many publications have shown HE4 as the most promising biomarker. Moore et al. demonstrated that HE4 has high sensitivity and specificity as well as greater sensitivity than CA 125 in early stages [51]. HE4 is an example of how a new biomarker becomes a clinical reality, but its clinical value has been less assessed than CA 125; some aspects remain unclear and still need further study, as pointed out in a recent meta-analysis [50].

Although the use of these TMs for screening purposes is generally not recommended, the measurement of CA 125 and transvaginal ultrasonography are reasonable options for women at high risk of ovarian cancer (e.g. patients with suggestive symptoms and carriers of germline pathogenic variants in *BRCA1/2* genes), according to the American College of Obstetricians and Gynecologists (ACOG) and The National Institute for Health and Clinical Excellence (NICE) current guidelines [52–54]. This strategy is based on the fact that positive predictive value is low in general population due to the low incidence of EOC, which can result in a considerable number of false positives; but diagnostic efficacy could be improved if we apply TMs in high-risk groups.

In this scenario, the Risk of Ovarian Malignancy Algorithm (ROMA) which combines CA 125 and HE4 along with menopause status, has been proposed to estimate the ovarian cancer risk in patients with adnexal masses [55]. The aim is to identify high-risk patients to be referred to a gynecologic oncologist for further testing as well as reduce the number of unnecessary surgeries. In an exhaustive meta-analysis, HE4 measurement seems to be superior to CA 125 in terms of diagnostic performance for the identification of EOC in women with suspected gynecological disease [56]. Molina et al. concluded that HE4 is the TM of choice in EOC, with higher efficiency than CA 125 and ROMA algorithm. Moreover, ROMA may be used in those patients with HE4 negative and CA 125 positive results, increasing the TM utility in the diagnosis of pelvic masses [57]. But results are inconclusive, in a recent comparison, the performance of CA 125, HE4, ROMA and Copenhagen index (CPH-I) to preoperatively identify EOC or metastatic cancer in the ovary was evaluated. ROMA and CPH-I perform better than TMs alone to identify patients harboring EOC or metastasic cancer [58].

Colorectal cancer (CRC), the third most common cancer, accounts for approximately 1.9 million new cases and 0.9 million deaths per year globally. Even though CEA is the most studied TM, lack of sensitivity in early stages combined with the low prevalence of CRC in asymptomatic populations excludes its use for screening. Although CEA concentration is not sufficient for CRC diagnosis in the absence of confirmatory biopsy, it should be evaluated before surgery, as baseline levels add prognosis information. Preoperative serum CEA concentration has been described as an independent predictor of overall survival across all stages [59], and postoperative increased levels also suggest a worse outcome [60].

Role of tumor markers in the follow-up of patients with cancer

In general, clinical practice guidelines agree that the main clinical application of TM is during the follow-up of cancer patients. In this sense, the role of PSA in the management of PCa patients is well-established [61, 62]. In the management of advanced cancer patients, two scenarios must be considered: (1) the early detection of relapse after primary treatment and (2) monitoring therapy response in advanced disease.

In BC, the usefulness of TMs in disease follow-up is also controversial. Most recommendations from oncology scientific societies are against measuring CA 15.3 or CEA during post-operative surveillance [63–65]. This is based on lack of evidence that early detection of recurrence by TMs, even months before radiological or clinical findings, has a clinical benefit. TMs diagnostic capacity depends on recurrence location, being low for local but higher for metastatic recurrences [66]. In this line, the EGTM recommends serial measurement of CA 15.3 and CEA for early detection of recurrence or metastatic disease in patients with BC and no evidence of disease, if it would alter clinical management [33]. In the current scenario, where more effective therapeutic options are available for metastatic patients and TMs continue to be requested it would be interesting to prospectively evaluate whether early detection of recurrences has an impact on patient outcomes. The recent consensus from the Spanish societies of laboratory medicine and medical oncology (SEQC-SEOM), suggests that their measurement should be limited to patients with a high risk of recurrence [34].

Conversely, the usefulness of CA 15.3 and CEA in the follow-up of advanced disease is supported, with different nuances, by most scientific societies [34]. It is important to

keep in mind that TMs results must be integrated with imaging tests and other relevant clinical information. Only in some situations, therapeutic decisions should be guided by TMs, for example when it is not possible to measure the degree of disease extension (e.g. non-measurable bone metastasis).

Concerning EOC, CA 125 is the most studied biomarker and its utility in follow-up is well known. The measurement of serum CA 125 during chemotherapy has long been used to evaluate treatment response, complementing imaging and clinical assessment. International clinical guidelines agree on its usefulness; however, there are different criteria to consider significant changes along the follow-up. Regarding the detection of recurrences, it has been suggested that HE4 outperforms CA 125 [67], but recent studies indicate that both TMs have a similar performance and, although HE4 adds information in some cases, CA 125 is the most reliable marker for disease monitoring [50].

Additionally, TMs have an important role in the non-invasive assessment of disease extension. Estimation of tumor burden is basic to select the best primary treatment for advanced EOC patients, but it is often challenging and usually performed through surgical procedures. As a recent study showed, both CA 125 and HE4 correlate to whole-body tumor burden assessed by PET/CT before the primary treatment, and HE4 has superior performance than CA 125 estimating peritoneal disease in patients with high-grade advanced OEC [68].

CEA is the TM of the highest value in the follow-up of patients with CRC, also strongly recommended by international guidelines. Different meta-analyses have shown that intensive follow-up including CEA monitoring is associated with significantly earlier detection of recurrence and has a significant impact on survival [69]. Thus, CEA monitoring is generally recommended after surgery, although there are discrepancies between different guidelines regarding the frequency of CEA testing [70–72]. Moreover, there is no definition of what constitutes a clinically significant increase in CEA concentration. Generally, differences of 25–30% between two values are considered significant, based on reference change values (RCV) [73]. CEA is also the marker of choice for monitoring treatment response and early detection of progression in patients with metastatic disease. Persistently increasing concentrations above baseline suggest progressive disease even in the absence of corroborating radiographs [74]. Thus, it is crucial to correctly interpret and confirm any increase, with special caution during the first 4–6 weeks of treatment, because transient elevations in absence of progression can occur.

Circulating biomarkers for precision oncology

Precision oncology seeks to achieve a more personalized and effective treatment of patients according to tumor characteristics. The improvements in molecular biology techniques and the identification of key driver genes have allowed the scientific community to go one step further in the knowledge of cancer molecular bases. This scenario has facilitated the approval of targeted therapies that are more effective and have demonstrated better patient outcomes than standard chemotherapy. The characterization of tumors through analysis of prognostic and predictive biomarkers improves patient stratification and allows the identification of candidates for targeted therapies. Consequently, therapeutic decisions are ever more dependent on tumor molecular profiling and have a multidisciplinary approach, leading to a paradigm shift in the management of most cancers.

The current challenge in applying personalized oncology is the limited availability and quality of specimens for molecular analyses. Tumor tissue is still the gold standard for molecular analysis, and tissue biopsy has drawbacks such as invasiveness, partial representation of intratumoral and intermetastatic genetic heterogeneity, or unfeasibility for longitudinal monitoring. To overcome these limitations, multiple studies focused on discover new non-invasive biomarkers to characterize tumor-specific signatures based on multi-omics analysis that combines genomic, transcriptomic, and proteomic data [75].

Liquid biopsy has emerged as a minimally invasive diagnostic tool to analyze tumoral genetic biomarkers released into circulation [76]. It demands less time and costs for sample obtaining (mainly peripheral blood) than tissue biopsy, captures better the genetic heterogeneity, and gives a dynamic picture of the tumor molecular landscape. This concept englobes different analytes such as circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), RNA (mRNA and microRNA), and extracellular vesicles (EV). The main disadvantage is lack of standardization of methodologies, necessary for use in clinical practice. Recent publications in CCLM provide an update on this field, concerning issues that still need to be addressed and future perspectives and focusing particularly on frequent neoplasms, such as lung, breast and prostate cancer.

The implementation of liquid-biopsy biomarkers, particularly (ctDNA), has had a relevant impact on the management of patients with advanced non-small cell lung

cancer (NSCLC), in which tumor genotyping is the standard of care. The analysis of activating mutations in *EGFR* gene is highly recommended because they are the most common druggable genetic alterations in lung adenocarcinomas and targeted treatment with EGFR-tyrosine kinase inhibitors (TKIs) has demonstrated improved survival. Unfortunately, it is not possible to obtain tumor tissue in up to 60% of patients with LC [77]. ctDNA measurement in blood and other fluids is an adequate source for *EGFR* testing [78], backed by oncology guidelines, to select treatment and also during the treatment monitoring to identify resistance mutations. The analysis of *EGFR* mutations in plasmatic ctDNA through cobas® EGFR Mutation Test v2 was the first liquid-biopsy test approved by the FDA in 2016, but recently, multiple pan-cancer panels based on next-generation sequencing (NGS) obtained FDA approval. The analysis of ctDNA is also useful during the follow-up of patients treated with TKIs, for early identification of resistance mutations (T790M). The correlation between TMs and molecular features has been scarcely studied, and although recent studies show that the combination of conventional TMs and ctDNA could be a promising strategy, further studies are needed to elucidate the relationship between serum TM and gene mutations [79].

BC is another successful example of precision oncology application, as the better comprehension of BC molecular profiles has improved tumor characterization and also prognosis estimation. Nowadays, BC is classified into four molecular subtypes: Luminal A, Luminal B, HER2-enriched and Basal-like. In particular, HER2 overexpression occurs in approximately 20–30% of patients and is associated with more aggressive behavior and poor prognosis [80]. The development of anti-HER2 targeted treatments has improved overall survival of patients with this subtype of BC [81]. In this scenario, the HER-2 immunoassay in serum (sHER2), which measures the extracellular domain (ECD) of the protein, was approved in 2000 by the FDA. Although sHER2 showed low sensitivity compared with conventional TMs, their combination (CEA, CA 15.3 and HER2) increased the globall sensitivity and is especially useful when conventional TMs are negative [82]. This newest TM is scarcely mentioned in guidelines, only the NACB supports its potential role for monitoring the disease in patients with advanced HER2-positive BC. Preliminary results pointed out their value as a prognostic and therapeutic predictive factor [83, 84], but further studies are needed.

Moreover, patients with BC present increased levels of cfDNA in plasma, which has been associated with more aggressive or metastatic disease [73, 85]. Changes in the specific fraction of cfDNA derived from the tumor, the circulating tumor DNA (ctDNA), were also evaluated as a biomarker of response [86]. The detection of ctDNA mutations and serial quantification of the variant allele frequency (%VAF) during the follow-up could play a crucial role to monitor response and also to detect resistance mutations [76, 87].

In PCa, the quantification of microRNas (miRNas) in serum or plasma has shown promising results for diagnosis and risk stratification, as they can be useful for the inclusion of patients in active surveillance. Different panels of specific circulating miRNas have been evaluated in PCa, however, since there is no homogeneity in the miRNa used, the precision obtained for this biomarker is variable. Best results were obtained using miR-141, miR-375 and miR-21 but more studies are needed to verify its usefulness. Secondly, preanalytical conditions and miRNAs isolation play an important role in miRNAs measurement. Because of that, miRNAs were isolated from urine after prostate massage, opening a promising way for the management of early PCa [88].

Conclusions

This review provides an overview of the successes and pitfalls of TMs in the management of patients with cancer. We summarized the main obstacles to their use and remarked on the value of clinical guidelines to improve the quality of care received by patients, saving potential harm derived from the inappropriate use of these tests.

For safe and correct use, it is essential to know the limitations and diagnostic efficacy of each TM in different clinical situations. TM results should be evaluated considering confounding factors, concomitant clinical conditions, and potential causes of false positives. With this aim, laboratory physicians have a key role investigating discordant results, as well as facilitating correct interpretation.

Furthermore, we reported recent changes in the clinical usefulness of circulating TMs, and the new role of PSA in a personalized PCa screening strategy. We also reviewed recent studies that seek more specific and sensitive TMs focusing on those who have reached the clinical routine. HE4 is a clear example of a novel biomarker that has become a reality, improving risk stratification of patients with suspected ovarian cancer.

Finally, we have focused on potential applications of circulating biomarkers in precision oncology. We summarized current challenges in this field, and how non-invasive approaches can overcome some limitations. We discussed

potential applications of liquid-biopsy biomarkers if real clinical integration is achieved. In this context, ctDNA have incorporated into clinical workflow, optimizing the management of cancer patients.

Research funding: None declared.
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.
Informed consent: Not applicable.
Ethical approval: Not applicable.

References

1. Trapé J, Filella X, Alsina-Donadeu M, Juan-Pereira L, Bosch-Ferrer À, Rigo-Bonnin R. Oncology Section of the Catalan Association of Clinical Laboratory Science. Increased plasma concentrations of tumour markers in the absence of neoplasia. Clin Chem Lab Med 2011;49:1605–20.
2. Gion M, Franceschini R, Rosin C, Trevisiol C, Peloso L, Zappa M, et al. An epidemiology-based model to estimate the rate of inappropriateness of tumor marker requests. Clin Chem Lab Med 2014;52:889–97.
3. Moreno-Campoy EE, Mérida-De la Torre FJ, Martos-Crespo F, Plebani M. Identifying risk in the use of tumor markers to improve patient safety. Clin Chem Lab Med 2016;54:1947–53.
4. Gion M, Trevisiol C, Rutjes AWS, Rainato G, Fabricio ASC. Circulating tumor markers: a guide to their appropriate clinical use: comparative summary of recommendations from clinical practice guidelines (PART 1). Int J Biol Markers 2016;31:332–67.
5. Gion M, Trevisiol C, Rutjes AWS, Rainato G, Fabricio ASC. Circulating tumor markers: a guide to their appropriate clinical use: comparative summary of recommendations from clinical practice guidelines (PART 2). Int J Biol Markers 2017;32:1–52.
6. Gion M, Trevisiol C, Rutjes AWS, Rainato G, Fabricio ASC. Circulating tumor markers: a guide to their appropriate clinical use. Comparative summary of recommendations from clinical practice guidelines (PART 3). Int J Biol Markers 2017;32:147–81.
7. Sturgeon CM, Duffy MJ, Stenman UH, Lilja H, Brünner N, Chan DW, et al. National Academy of Clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers. Clin Chem 2008;54:e11–79.
8. Sturgeon CM, Hoffman BR, Chan DW, Ch'ng SL, Hammond E, Hayes DF, et al. National Academy of clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in clinical practice: quality requirements. Clin Chem 2008;54:e1–10.
9. Filella X, Albaladejo MD, Allué JA, Castaño MA, Morell-Garcia D, Ruiz MÀ, et al. Prostate cancer screening: guidelines review and laboratory issues. Clin Chem Lab Med 2019;57:1474–87.
10. Stephan C, Köpke T, Semjonow A, Lein M, Deger S, Schrader M, et al. Discordant total and free prostate-specific antigen (PSA) assays: does calibration with WHO reference materials diminish the problem? Clin Chem Lab Med 2009;47:1325–31.
11. Boegemann M, Arsov C, Hadaschik B, Herkommer K, Imkamp F, Nofer JR, et al. Discordant prostate specific antigen test results despite WHO assay standardization. Int J Biol Markers 2018;33:275–82.
12. Passerini R, Riggio D, Salvatici M, Zorzino L, Radice D, Sandri MT. Interchangeability of measurements of CA 19-9 in serum with four frequently used assays: an update. Clin Chem Lab Med 2007;60:351–60.
13. Solsvik AE, Kristoffersen AH, Sandberg S, Gidske G, Stavelin AV, Eikeland J, et al. A national surveillance program for evaluating new reagent lots in medical laboratories. Clin Chem Lab Med 2022;60:351–60.
14. Stenman UH, Leinonen J, Hakama M, Knekt P, Aromaa A, Teppo L. Serum concentrations of prostate specific antigen and its complex with α1-antichymotrypsin before diagnosis of prostate cancer. Lancet 1994;344:1594–8.
15. Gann PH, Hennekens CH, Stampfer MJ. A prospective evaluation of plasma prostate-specific antigen for detection of prostatic cancer. JAMA 1995;273:289–94.
16. Loeb S, Roehl KA, Antenor JAV, Catalona WJ, Suarez BK, Nadler RB. Baseline prostate-specific antigen compared with median prostate-specific antigen for age group as predictor of prostate cancer risk in men younger than 60 years old. Urology 2006;67:316–20.
17. Lilja H, Ulmert D, Björk T, Becker C, Serio AM, Nilsson JÅ, et al. Long-term prediction of prostate cancer up to 25 years before diagnosis of prostate cancer using prostate kallikreins measured at age 44 to 50 years. J Clin Oncol 2007;25:431–6.
18. Vickers AJ, Cronin AM, Bjork T, Manjer J, Nilsson PM, Dahlin A, et al. Prostate specific antigen concentration at age 60 and death or metastasis from prostate cancer: case-control study. BMJ 2010;341:c4521.
19. Preston MA, Batista JL, Wilson KM, Carlsson SV, Gerke T, Sjoberg DD, et al. Baseline prostate-specific antigen levels in midlife predict lethal prostate cancer. J Clin Oncol 2016;34:2705–11.
20. Kovac E, Carlsson SV, Lilja H, Hugosson J, Kattan MW, Holmberg E, et al. Association of baseline prostate-specific antigen level with long-term diagnosis of clinically significant prostate cancer among patients aged 55 to 60 years. JAMA Netw Open 2020;3:e1919284.
21. Gilligan TD, Seidenfeld J, Basch EM, Einhorn LH, Fancher T, Smith DC, et al. American society of clinical oncology clinical practice guideline on uses of serum tumor markers in adult males with germ cell tumors. J Clin Oncol 2010;28:3388–404.
22. Molina R, Bosch X, Auge JM, Filella X, Escudero JM, Molina V, et al. Utility of serum tumor markers as an aid in the differential diagnosis of patients with clinical suspicion of cancer and in patients with cancer of unknown primary site. Tumor Biol 2012;33:463–74.
23. Duffy MJ. Biomarkers for prostate cancer: prostate-specific antigen and beyond. Clin Chem Lab Med 2020;58:326–39.
24. Ilic D, Djulbegovic M, Jung JH, Hwang EC, Zhou Q, Cleves A, et al. Prostate cancer screening with prostate-specific antigen (PSA) test: a systematic review and meta-analysis. BMJ 2018;362:k3519.

25. Gandaglia G, Albers P, Abrahamsson PA, Briganti A, Catto JWF, Chapple CR, et al. Structured population-based prostate-specific antigen screening for prostate cancer: the European Association of Urology position in 2019. Eur Urol 2019;76:142–50.
26. Van Poppel H, Hogenhout R, Albers P, van den Bergh RCN, Barentsz JO, Roobol MJ. Early detection of prostate cancer in 2020 and beyond: facts and recommendations for the European Union and the European Commission. Eur Urol 2021;79:327–9.
27. Van Poppel H, Roobol MJ, Chapple CR, Catto JWF, N'Dow J, Sønksen J, et al. Prostate-specific antigen testing as part of a risk-adapted early detection strategy for prostate cancer: European Association of Urology position and recommendations for 2021. Eur Urol 2021;80:703–11.
28. Hugosson J, Roobol MJ, Månsson M, Tammela TLJ, Zappa M, Nelen V, et al. A 16-yr follow-up of the European randomized study of screening for prostate cancer. Eur Urol 2019;76:43–51.
29. European Comission. Proposal for a COUNCIL RECOMMENDATION on strengthening prevention through early detection: a new EU approach on cancer screening replacing Council Recommendation 2003/878/EC, 2022. Available from: https://health.ec.europa.eu/publications/proposal-council-recommendation-cr-strengthening-prevention-through-early-detection-new-approach_en.Wicaksana.
30. European Comission. Annex to the proposal for a Council Recommendation on strengthening prevention through early detection: a new EU approach on cancer screening replacing Council Recommendation 2003/878/EC, 2022. Available from: https://health.ec.europa.eu/publications/annex-proposal-council-recommendation-cr-strengthening-prevention-through-early-detection-new_en.
31. Duffy MJ, McDermott EW, Crown J. Blood-based biomarkers in breast cancer: from proteins to circulating tumor cells to circulating tumor DNA. Tumor Biol 2018;40: 101042831877616.
32. Molina R, Auge JM, Farrus B, Zanón G, Pahisa J, Muñoz M, et al. Prospective evaluation of carcinoembryonic antigen (CEA) and carbohydrate antigen 15.3 (CA 15.3) in patients with primary locoregional breast cancer. Clin Chem 2010;56:1148–57.
33. Molina R, Barak V, van Dalen A, Duffy MJ, Einarsson R, Gion M, et al. Tumor markers in breast cancer. European group on tumor markers recommendations. Tumor Biol 2005;26:281–93.
34. Ayala de la Peña F, Ortiz-Muñoz B, Quintanar-Verdúguez T, Santotoribio JD, de la Cruz S, Trapé-Pujol J, et al. Consensus of the Spanish society of laboratory medicine and the Spanish society of medical oncology on the methodology and criteria for evaluation of circulating tumour markers in breast cancer. Clin Transl Oncol 2021;23:1272–80.
35. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2019;30:1194–220.
36. Gradishar WJ, Anderson BO, Abraham J, Aft R, Agnese D, Allison KH, et al. Breast cancer, version 3.2020, NCCN clinical practice guidelines in oncology. J Natl Compr Cancer Netw 2020;18:452–78.
37. Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, et al. American society of clinical oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2007;25:5287–312.
38. Daniele A. Clinical usefulness of cancer antigen 15-3 in breast cancer patients before and after surgery. Open Breast Cancer J 2013;5:1–6.
39. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med 2011;365:395–409.
40. de Koning HJ, van der Aalst CM, de Jong PA, Scholten ET, Nackaerts K, Heuvelmans MA, et al. Reduced lung-cancer mortality with volume CT screening in a randomized trial. N Engl J Med 2020;382:503–13.
41. Seijo LM, Peled N, Ajona D, Boeri M, Field JK, Sozzi G, et al. Biomarkers in lung cancer screening: achievements, promises, and challenges. J Thorac Oncol 2019;14:343–57.
42. Doseeva V, Colpitts T, Gao G, Woodcock J, Knezevic V. Performance of a multiplexed dual analyte immunoassay for the early detection of non-small cell lung cancer. J Transl Med 2015;13:55.
43. Mazzone PJ, Wang XF, Han X, Choi H, Seeley M, Scherer R, et al. Evaluation of a serum lung cancer biomarker panel. Biomark Insights 2018;13:117727191775160.
44. Cohen JD, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science 2018;359:926–30.
45. Molina R, Marrades RM, Augé JM, Escudero JM, Viñolas N, Reguart N, et al. Assessment of a combined panel of six serum tumor markers for lung cancer. Am J Respir Crit Care Med 2016; 193:427–37.
46. Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman JR, Bharat A, et al. Non–small cell lung cancer, version 3.2022, NCCN clinical practice guidelines in oncology. J Natl Compr Cancer Netw 2022; 20:497–530.
47. European Group on. Tumors Markers. Tumour markers in lung cancer: EGTM recommendations. Anticancer Res 1999;19: 2817–9.
48. Callister MEJ, Baldwin DR, Akram AR, Barnard S, Cane P, Draffan J, et al. British Thoracic Society guidelines for the investigation and management of pulmonary nodules: accredited by NICE. Thorax 2015;70(2 Suppl):ii1–54.
49. Ferraro S, Panteghini M. Making new biomarkers a reality: the case of serum human epididymis protein 4. Clin Chem Lab Med 2019;57:1284–94.
50. Moore RG, Brown AK, Miller MC, Skates S, Allard WJ, Verch T, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. Gynecol Oncol 2008;108:402–8.
51. American College of Obstetricians and Gynecologists. Practice bulletin no. 182 summary: hereditary breast and ovarian cancer syndrome. Obstet Gynecol 2017;130:657–9.
52. Sturgeon CM, Duffy MJ, Walker G. The National Institute for Health and Clinical Excellence (NICE) guidelines for early detection of ovarian cancer: the pivotal role of the clinical laboratory. Ann Clin Biochem 2011;48:295–9.
53. Dullens B, de Putter R, Lambertini M, Toss A, Han S, Van Nieuwenhuysen E, et al. Cancer surveillance in healthy carriers of germline pathogenic variants in BRCA1/2: a review of secondary prevention guidelines. J Oncol 2020;2020:1–13.
54. Moore RG, McMeekin DS, Brown AK, DiSilvestro P, Miller MC, Allard WJ, et al. A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. Gynecol Oncol 2009;112:40–6.

55. Ferraro S, Braga F, Lanzoni M, Boracchi P, Biganzoli EM, Panteghini M. Serum human epididymis protein 4 vs carbohydrate antigen 125 for ovarian cancer diagnosis: a systematic review. J Clin Pathol 2013;66:273–81.
56. Molina R, Escudero JM, Augé JM, Filella X, Foj L, Torné A, et al. HE4 a novel tumour marker for ovarian cancer: comparison with CA 125 and ROMA algorithm in patients with gynaecological diseases. Tumor Biol 2011;32:1087–95.
57. Carreras-Dieguez N, Glickman A, Munmany M, Casanovas G, Agustí N, Díaz-Feijoo B, et al. Comparison of HE4, CA125, ROMA and CPH-I for preoperative assessment of adnexal tumors. Diagnostics 2022;12:226.
58. Becerra AZ, Probst CP, Tejani MA, Aquina CT, González MG, Hensley BJ, et al. Evaluating the prognostic role of elevated preoperative carcinoembryonic antigen levels in colon cancer patients: results from the national cancer database. Ann Surg Oncol 2016;23:1554–61.
59. Konishi T, Shimada Y, Hsu M, Tufts L, Jimenez-Rodriguez R, Cercek A, et al. Association of preoperative and postoperative serum carcinoembryonic antigen and colon cancer outcome. JAMA Oncol 2018;4:309.
60. Mottet N, van den Bergh RCN, Briers E, Van den Broeck T, Cumberbatch MG, De Santis M, et al. EAU-EANM-ESTRO-ESUR-SIOG guidelines on prostate cancer—2020 update. Part 1: screening, diagnosis, and local treatment with curative intent. Eur Urol 2021;79:243–62.
61. Cornford P, van den Bergh RCN, Briers E, Van den Broeck T, Cumberbatch MG, De Santis M, et al. EAU-EANM-ESTRO-ESUR-SIOG guidelines on prostate cancer. Part II—2020 update: treatment of relapsing and metastatic prostate cancer. Eur Urol 2021;79:263–82.
62. Henry NL, Somerfield MR, Dayao Z, Elias A, Kalinsky K, McShane LM, et al. Biomarkers for systemic therapy in metastatic breast cancer: ASCO guideline update. J Clin Oncol 2022;40: 3205–21.
63. Van Poznak C, Somerfield MR, Bast RC, Cristofanilli M, Goetz MP, Gonzalez-Angulo AM, et al. Use of biomarkers to guide decisions on systemic therapy for women with metastatic breast cancer: American society of clinical oncology clinical practice guideline. J Clin Oncol 2015;33:2695–704.
64. Gennari A, André F, Barrios CH, Cortés J, de Azambuja E, DeMichele A, et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. Ann Oncol 2021;32:1475–95.
65. Sölétormos G, Petersen PH, Dombernowsky P. Assessment of CA 15.3, CEA and TPA concentrations during monitoring of breast cancer. Clin Chem Lab Med 2000;38: 453–63.
66. Piovano E, Attamante L, Macchi C, Cavallero C, Romagnolo C, Maggino T, et al. The role of HE4 in ovarian cancer follow-up: a review. Int J Gynecol Cancer 2014;24:1359–65.
67. Glickman A, Paredes P, Carreras-Diéguez N, Niñerola-Baizán A, Gaba L, Pahisa J, et al. Evaluation of patients with advanced epithelial ovarian cancer before primary treatment: correlation between tumour burden assessed by [18F]FDG PET/CT volumetric parameters and tumour markers HE4 and CA125. Eur Radiol 2022; 32:2200–8.
68. Renehan AG. Impact on survival of intensive follow up after curative resection for colorectal cancer: systematic review and meta-analysis of randomised trials. BMJ 2002;324:813.
69. Glynne-Jones R, Wyrwicz L, Tiret E, Brown G, Rödel C, Cervantes A, et al. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2017;28:iv22–40.
70. Meyerhardt JA, Mangu PB, Flynn PJ, Korde L, Loprinzi CL, Minsky BD, et al. Follow-up care, surveillance protocol, and secondary prevention measures for survivors of colorectal cancer: American Society of Clinical Oncology clinical practice guideline endorsement. J Clin Oncol 2013;31:4465–70.
71. Argilés G, Tabernero J, Labianca R, Hochhauser D, Salazar R, Iveson T, et al. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2020;31:1291–305.
72. Sturgeon C. Practice guidelines for tumor marker use in the clinic. Clin Chem 2002;48:1151–9.
73. American Society of Clinical Oncology. 2006 Update of ASCO recommendations for the use of tumor markers in gastrointestinal cancer. J Oncol Pract 2006;2:314–6.
74. Heitzer E, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. Nat Rev Genet 2019;20:71–88.
75. Pinzani P, D'Argenio V, Del Re M, Pellegrini C, Cucchiara F, Salvianti F, et al. Updates on liquid biopsy: current trends and future perspectives for clinical application in solid tumors. Clin Chem Lab Med 2021;59:1181–200.
76. Marchetti A, Palma JF, Felicioni L, De Pas TM, Chiari R, Del Grammastro M, et al. Early prediction of response to tyrosine kinase inhibitors by quantification of EGFR mutations in plasma of NSCLC patients. J Thorac Oncol 2015;10:1437–43.
77. Aledo-Castillo De JMG, Arcocha A, Victoria I, Isabel A. Molecular characterization of advanced non-small cell lung cancer patients by cfDNA analysis: experience from routine laboratory practice. J Thorac Dis 2021;13:1658–70.
78. Chen Z, Liu L, Zhu F, Cai X, Zhao Y, Liang P, et al. Dynamic monitoring serum tumor markers to predict molecular features of EGFR-mutated lung cancer during targeted therapy. Cancer Med 2022;11:3115–25.
79. Waks AG, Winer EP. Breast cancer treatment. JAMA 2019;321:288.
80. Burris HA, Rugo HS, Vukelja SJ, Vogel CL, Borson RA, Limentani S, et al. Phase II study of the antibody drug conjugate trastuzumab-DM1 for the treatment of human epidermal growth factor receptor 2 (HER2)–positive breast cancer after prior HER2-directed therapy. J Clin Oncol 2011;29:398–405.
81. Molina R, Jo J, Zanón G, Filella X, Farrus B, Muñoz M, et al. Utility of C-erbB-2 in tissue and in serum in the early diagnosis of recurrence in breast cancer patients: comparison with carcinoembryonic antigen and CA 15.3. Br J Cancer 1996;74:1126–31.
82. Molina R, Escudero JM, Muñoz M, Augé JM, Filella X. Circulating levels of HER-2/neu oncoprotein in breast cancer. Clin Chem Lab Med 2012;50:5–21.
83. Zhang P, Xiao J, Ruan Y, Zhang Z, Zhang X. Monitoring value of serum HER2 as a predictive biomarker in patients with metastatic breast cancer. Cancer Manag Res 2020;12:4667–75.
84. Guo Q, Hua Y. The assessment of circulating cell-free DNA as a diagnostic tool for breast cancer: an updated systematic review

and meta-analysis of quantitative and qualitative ssays. Clin Chem Lab Med 2021;59:1479–500.
85. Deligezer U, Eralp Y, Akisik EE, Akisik EZ, Saip P, Topuz E, et al. Size distribution of circulating cell-free DNA in sera of breast cancer patients in the course of adjuvant chemotherapy. Clin Chem Lab Med 2008;46:311–7.
86. Page K, Guttery DS, Fernandez-Garcia D, Hills A, Hastings RK, Luo J, et al. Next generation sequencing of circulating cell-free DNA for evaluating mutations and gene amplification in metastatic breast cancer. Clin Chem 2017;63:532–41.
87. Filella X, Foj L. miRNAs as novel biomarkers in the management of prostate cancer. Clin Chem Lab Med 2017;55:715–36.
88. Stieber P, Hatz R, Holdenrieder S, Molina R, Nap M, von Pawel J, et al. National Academy of Clinical Biochemistry Guidelines for the use of tumor markers in lung cancer, 2006; Published Online. Available from http://www.nacb.org/lmpg/tumor_LMPG_draft_PDF.stm.