NIH Public Access
Author Manuscript
Expert Opin Med Diagn. Author manuscript; available in PMC 2010 November 1.

Published in final edited form as:
Expert Opin Med Diagn. 2009 November 1; 3(6): 597–605. doi:10.1517/17530050903266830.

Early Detection of Cancer: Immunoassays for Plasma Tumor Markers

Danni L. Meany, **Lori J. Sokoll**, and **Daniel W. Chan***
Center for Biomarker Discovery, Department of Pathology, Johns Hopkins University, Baltimore, MD 21287, USA

Abstract

Background—Plasma tumor biomarkers are widely used clinically for monitoring response to therapy and detecting cancer recurrence. However, only a limited number of them have been effectively used for the early detection of cancer.

Objective—To review plasma tumor markers used clinically for the early detection of cancer and to provide expert opinion about future directions.

Methods—Literature review, as well as our expert opinion, of plasma tumor markers that have been widely accepted for the early detection of cancer.

Results—In the United States, only prostate specific antigen (PSA), cancer antigen 125 (CA125), and alpha-fetoprotein (AFP) have been clinically used for the early detection of prostate, ovarian, and liver cancers, respectively. Both analytical and clinical issues related to the use of these three markers were discussed.

Conclusion—Few plasma tumor markers have been used effectively for the early detection of cancer, mainly due to their limited sensitivity and/or specificity. Multiple approaches have been developed to improve the clinical performance of tumor markers for the early detection of cancer. Metrological traceability and antibody specificity are important issues to ensure comparability of immunoassays for the measurement of plasma tumor markers.

Keywords

plasma; tumor marker; early detection; cancer

1. Introduction

Currently, one in four deaths in the United States is due to cancer[1]. Despite significant funding in cancer research, poor survival is common for advanced disease due to the lack of effective treatment options[2]. The 5-year relative survival rates among patients who are diagnosed with either advanced lung, colorectal, or breast cancer are only 3%, 10%, and 27%, respectively [1]. By contrast, survival is much better when cancers are diagnosed at an early stage. The 5-year relative survival rates among patients diagnosed with localized lung, colorectal or breast cancers are significantly higher at 50%, 90%, and 98%, respectively[1]. Based on these

***Corresponding Author:** CRB2 Building Room3M05, Johns Hopkins University, 1550 Orleans St., Baltimore, MD 21231, USA dchan@jhmi.edu.

Declaration of interest
The authors state no conflict of interest and have received no payment in preparation of this manuscript.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

statistics, diagnosing cancer at an early stage before they metastasize and become incurable - the concept of early detection-will have a significant impact on cancer survival.

Various tests and imaging tools have been recommended for early detection of cancer. Examples are annual mammograms for the early detection of breast cancer, fecal occult blood test (FOBT) and colonoscopy for colorectal cancer, prostate specific antigen (PSA) and digital rectal exam (DRE) for prostate cancer, and *Papanicolaou* smear test for cervical cancer[3]. An important approach to early detection is to measure plasma tumor markers since plasma is readily available and most human tumors produce a variety of factors which, if they pass into the blood, may serve as plasma tumor markers. These biomarkers, either produced by the tumor itself or in response to the tumor, can be used to determine the presence of cancer based on measurement in blood samples.

An ideal plasma tumor marker for the early detection of cancer should have several properties. It should be sensitive enough to detect small tumors at an early stage. It should be specific for a given type of cancer, not present in non-cancer (healthy and benign) conditions, and released only in response to cancer. Currently, most plasma tumor markers are neither sensitive nor specific enough for this purpose. As a result, only three plasma tumor markers are typically used clinically for early detection in the United States: PSA, CA-125 and AFP. PSA is the only marker that has been approved by the United States Food and Drug Administration (FDA) for early detection (in conjunction with digital rectal examination). Nevertheless, CA-125 and alpha fetoprotein (AFP) are used in populations at high risk for cancer. CA-125 is used with transvaginal ultrasound for ovarian cancer screening in women at high risk and AFP is used for screening hepatocellular carcinoma (HCC) in populations at high risk. In this review, we will only discuss these three tumor biomarkers, which are measured using immunoassays, for the early detection of cancer. We use the terms plasma and serum interchangeably. In fact, most immunoassays for tumor markers use serum, not plasma. Although these three markers can also be used for evaluating cancer progression after initial therapy and monitoring response to treatment, these clinical applications will be not discussed.

2. PSA

PSA is a 237-amino acid single chain serine protease. Synthesized in the ductal epithelium and prostatic acini and secreted into the lumina of the prostatic ducts, PSA is a major protein in both the prostatic lumen and seminal plasma. Although PSA reaches the blood after diffusion from luminal cells through the epithelial basement membrane and prostatic stroma, under normal physiological conditions, PSA concentrations in the blood are low[4]. The mechanism of PSA elevation in blood is not clear. It is hypothesized that prostatic diseases (e.g., prostate cancer, prostatitis, benign prostatic hyperplasia (BPH), and prostatic intraepithelial neoplasia) breach the barriers (e.g., epithelial basement membrane and prostatic stroma) between the prostate lumen and capillaries[5]. As a result, the elevated PSA levels in blood can be used as a marker for prostate cancer. However, because other prostatic diseases could also elevate PSA in blood, PSA is not cancer-specific.

PSA has both free and complexed forms. Released into blood, PSA binds with major extracellular antiproteases such as alpha-1-antichymotrypsin (ACT) and alpha-2-macroglobulin (AMG) and forms PSA-ACT and PSA-AMG complexes. Although molar concentrations of these antiproteases are 1,000 fold higher than that of PSA, 5–45% of PSA in blood is still in free form, unreactive with the excessive ACT and AMG.

Existence of both free and complexed forms of PSA in blood has important implications for the design of immunoassays. First, assays for different forms of PSA (e.g., total, free, and complexed) have been developed. The total PSA assay measures both free and complexed PSA. Because AMG engulfs PSA, blocking access of PSA to antibodies[6], the total PSA assay

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

essentially measures only free PSA and the PSA-ACT complex. Second, total, free, and complexed assays use PSA antibodies with different specificities.

The design of these immunoassays has important implications for comparability. In fact, not all the PSA immunoassays-either total or free- give the same results. The comparability issue lies on two fundamental principles of immunoassays: metrological traceability and antibody specificity[7]. Metrological traceability requires use of International Standards for PSA so that analytical results from different PSA immunoassays can be traced back to these standards. Historically, total PSA assays were calibrated using the conventional Hybritech standards from Beckman Coulter. These standards are different from the WHO standards (96/670: 90% PSA-ACT complex and 10% free PSA and 96/668: free PSA only)[8,9]. Therefore, the clinical decision level of total PSA at 4 ng/mL established for the early detection of prostate cancer using the Hybritech standards is different than that of 3 ng/mL established using these WHO standards[10].

While metrological traceability reduces immunoassay discrepancies, it will not achieve the goal of interchangeability unless antibody specificity is also considered. Antibody specificity determines not only antigen binding sites on the PSA molecule but also the binding affinity. Some antibodies bind more strongly to either free PSA or the PSA-ACT complex. Based on their relative abilities to detect free and the PSA-ACT complex, total PSA assays among different manufacturers are divided into two groups: non-equimolar and equimolar. Non-equimolar assays may respond to free PSA more strongly than to the PSA-ACT complex or vice-versa. When proportions of free PSA increase, non-equimolar assays overestimate free PSA and thus overestimate the total PSA concentration. Equimolar total PSA assays, on the other hand, detect free PSA and the PSA-ACT complex equally, unbiased by their proportions [11]. Because of the existence of different proportions of free PSA in blood, equimolarity is essential to compare total PSA results among different laboratories and assay manufacturers [7].

Since analytical results of PSA assays affect clinical outcomes of patients, efforts have been made to improve the comparability of PSA immunoassays. According to the 2009 College of American Pathologists (CAP) survey, the variation of total PSA levels among different manufacturers is 10%, greatly reduced from 62% in the 1997 CAP survey[12]. Nevertheless, this variation among different immunoassays still exists. Therefore, keeping in mind that assay variation may be one potential source of discrepant total PSA results will be helpful in interpretation of patients' results.

2.1 Total PSA for early detection

Recently published results from two randomized controlled trials for evaluating the efficacy of PSA screening using serum total PSA measurements and/or digital rectal examination (DRE) has spurred speculation on whether PSA should be used for the early detection of prostate cancer[13,14]. One found that although screening does diagnose more prostate cancer, finding those cancers early does not reduce the risk of dying from the disease after 7 to 10 years of follow-up[14]. The other showed that screening reduced the mortality rate by 20% but was associated with a high risk of overdiagnosis[13]. Thus, the controversy of PSA screening for prostate cancer continues[15].

The total PSA test has been recommended by the American Cancer Society for the early detection of prostate cancer in combination with DRE annually, starting at age 50 years, for men who have a life expectancy of at least ten years[3]. When total PSA levels and/or DRE results are abnormal, prostate biopsy may be performed and used to diagnose cancer. Total PSA levels in serum have been classified into three categories: 0–4.0 ng/mL, 4.0–10.0 ng/mL, and > 10.0ng/mL. The risk of prostate cancer and the necessity of a biopsy are assessed based

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

on these categories. When the total PSA level is less than 4.0 ng/mL, the risk of cancer is considered to be low. However, a recent study showed that up to 27% of men with the total PSA in the 3.1–4.0 ng/mL range had cancer[16]. When the total PSA is greater than 10 ng/mL, 40–50% of patients have cancer[17]; and biopsy is typically performed. When the total PSA level is in the 4.0–10.0 ng/mL range, however, only 25–35% of patients have cancer based on biopsy[17]; therefore this range is referred as the diagnostic gray zone of total PSA[5].

As discussed before, elevation of PSA in serum is prostate-specific, but not cancer-specific. It rises in the context of cancer and other non-malignant pathological changes in prostate. Multiple approaches have been attempted to improve the cancer specificity of PSA. These approaches include measurements of free PSA, complexed PSA, and free PSA isoforms, calculations of PSA density and velocity, and use of age-specific PSA cut offs. Free PSA and PSA velocity are the two most successful approaches among them. Here we will review the impact that these two approaches have had on the early detection of prostate cancer.

2.2 Percent Free PSA for early detection

Percent free PSA (free PSA/total PSA ×100) is recommended for the risk assessment of prostate cancer when total PSA concentrations are between 4–10 ng/mL. A % free PSA of >25% indicates a low risk of cancer (e.g. probability = 8%) whereas a %free PSA of <10% suggests a high risk (e.g. probability = 56%)[5]. Percent free PSA has significantly increased efficacy of the early detection of prostate cancer by reducing unnecessary biopsies. A cut off of 25% detected 95% of cancers and reduced the biopsy rate by 20% when total PSA levels were between 4–10 ng/mL[18].

However, percent free PSA is not perfect. Both analytical and biological problems exist. The analytical problems are several-fold. First, because percent free PSA is calculated using the free PSA to total PSA ratio, problems exist for making a generalized recommendation on the cut off of %free PSA because of the lack of comparability of free PSA immunoassays among manufacturers. Examination of the 2009 CAP survey of PSA ratio indicated that %free PSA results between methods on the same survey material ranged from 20% to 33%. As a result, %free PSA cut offs are method-dependent. It is recommended that the percent free PSA be calculated using free and total PSA immunoassays from the same manufacturer. Second, lack of a commonly accepted pre-analytical sample handling procedure affects in vitro stability of different forms of PSA and therefore the %free PSA cut offs[19,20]. After storage for 7 days at 4°C, serum of patients with prostate cancer or BPH, and elderly men without known prostate disease showed significant decreases of percent free PSA[20]. Therefore, samples that are to be retained for longer than 24 hours should be frozen and samples stored for extended periods should be kept at −70 °C[19]. Interpretation of the percent free PSA results should be done with caution if the sampling and storage conditions are unknown. Lastly, high analytical precision is important for free PSA, especially at low levels, to reduce overlaps between men with and without cancer.

Biologically speaking, measurements of free and total PSA should be avoided during the first 48 –72 hours after prostatic manipulation, because it increases free PSA. In addition, differences in percent free PSA between men with and without cancer diminish with increasing total prostate volume[21]. When prostate volume is less than 35cc, a cut off of 14% is applied; when prostate volume is larger than 35cc, a cut off of 25% should be used[22].

2.3 PSA velocity

PSA velocity calculates changes in total PSA levels over time. The rationale for PSA velocity is based on the assumption that prostate cancer increases PSA levels in blood faster than other

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

benign prostatic conditions do. There are many ways to calculate PSA velocity.[23] Carter *et al* used the equation as follows[24]:

$$\text{PSA velocity} = \frac{1}{2} \times \frac{(PSA2 - PSA1)}{Time1} + \frac{1}{2} \times \frac{(PSA3 - PSA2)}{Time2}$$

PSA1 is the first total PSA measurement, PSA2 the second, and PSA3 the third in a two-year period or at least 12 to 18 months apart. Time 1 and Time 2 are differences in time expressed in years.

PSA velocity has significantly improved the cancer specificity of PSA especially in differentiating between cancer and BPH[25]. In addition, PSA velocity helps identify those men who would benefit from prostate cancer diagnosis at PSA levels associated with curable disease. When PSA levels are in the range of 2–4 ng/mL in which most men have curable disease, a PSA velocity greater than 0.35 ng/mL/year (10 to 15 years before diagnosis) was associated with a five-fold risk of death from prostate cancer 15 or more years later compared to a PSA velocity of 0.35 ng/mL/year or less[26]. Greater PSA velocity cut offs (e.g. 0.75 ng/mL/year) might be more appropriate for risk assessment in men with PSA levels in the range of 4–10 ng/mL[27].PSA velocity is unlikely to be incrementally useful among men with PSA levels greater than 10 ng/mL[28].

Caveats of using PSA velocity are several. First, intra-individual day-to-day PSA levels vary significantly. A total PSA increase of less than 20–46% is more likely due to biological and assay variations. Second, PSA elevations (e.g., due to inflammation) may affect velocity calculations performed over short periods of time[29]. So PSA velocity should be calculated over a two-year period or with PSA measurements at least 12 to 18 months apart. Finally, the same PSA assays should be used for the velocity calculation because of the incomparability of PSA results among different manufacturers. Despite these limitations, PSA velocity is still useful in assessing the need for a prostate biopsy in patients with a total PSA below 10 ng/mL and unremarkable DRE.

2.4 Other approaches

Other approaches to improve the cancer specificity of PSA include PSA density, age specific PSA reference ranges, complexed PSA, and free PSA isoforms. PSA density divides total PSA levels by the prostate volume determined by transrectal ultrasound (TRUS). It is based on the observation of a positive relationship between PSA levels in blood and prostate volumes. However, PSA density is not useful in the early detection of prostate cancer because (i) the volume determination by TRUS is examiner-dependent and (ii) the ratio of stroma and epithelial tissues is different in individual prostates. This ratio affects PSA density since only prostate epithelial tissues produce PSA[30].

The rationale of age-specific reference ranges for PSA is that (i) the prostate increases in size with age and (ii) younger men have lower normal PSA levels than older men[31]. Therefore, age specific PSA could improve the sensitivity of prostate cancer detection in younger aged men and spare unnecessary biopsies in older men. However, recent studies that compared age specific PSA cut offs to the standard cut off of 4 ng/mL showed conflicting results: one showed that age specific cut offs increased cancer detection by 8% in men below age 59 and spared 21% biopsies in men older than 60 while missing 4% of organ-confined cancers[32]; the other showed that the standard cut off of 4ng/mL was optimal for all age groups[33].

Prostate cancer influences the proportions of the PSA-ACT complex in blood. Recent development of a FDA-approved complexed PSA (cPSA) immunoassay has facilitated

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

studying the early detection of prostate cancer using the PSA-ACT complex. Although many of the studies conducted to date have shown improved cancer specificity of cPSA over total PSA, there is no improvement over percent free PSA[34].

Free PSA has distinct isoforms, analysis of which has shown encouraging results for improving the cancer specificity of PSA. BPSA is an internally cleaved, nicked, or multi-chain free PSA that is cleaved at Lys_{182}-Ser_{183} positions. A BPSA immunoassay demonstrated significant levels of BPSA in serum of men with BPH but undetectable in normal men. [−2]proPSA is a truncated precursor form of free PSA that has two additional amino acids in a pro-leader sequence. Recently an automated immunoassay for [−2]proPSA was developed and employed in a multi-center study, which showed that %[−2]proPSA ([−2]proPSA /free PSA) was a better predictor of prostate cancer than percent free PSA, particularly in the 2–10ng/mL total PSA range[35]. Despite these promising results, further evaluation and additional characterization of these free isoforms is needed to assess their potential for the early detection of prostate cancer.

3. CA125

CA125 is a heavily glycosylated mucin (MUC16). Recent cloning of the peptide core of CA125 revealed that it has a 156-amino-acid tandem repeat region in the N-terminus, and a possible transmembrane region and a tyrosine phosphorylation site in the C-terminus[36]. Due to the lack of purified CA125 for standardization, all CA125 immunoassay results are expressed in U (units)/mL. Two major antigenic domains on CA125 are characterized as OC125-like and M11-like[37]. The first generation of CA125 assay used only the OC125 antibody for both capture and detection. The second generation CA125 assays use the M11 antibody for capture and OC125 for detection. As a result of utilizing two distinct antibodies, the second generation assay has improved inter-assay precision and linearity, and diminished high-dose hook effect. Since then, automated immunoassays for CA125 have been developed by different manufacturers. The Cut off for the majority of these assays is 35 U/mL, established from the distribution of CA125 results in healthy women. Despite the same cut offs, these immunoassays can give different results on the same specimen therefore their results are not interchangeable. Laboratory reports should indicate the specific CA125 assay used. Patients should be serially monitored using the same assay.

3.1 CA125 for early detection of ovarian cancer

Because the prevalence of epithelial ovarian cancer in the post-menopausal population is low (e.g., ~ 1 in 2500), an effective screening modality must have a sensitivity greater than 75% and a specificity greater than 99.6% to attain a positive predictive value (PPV) of 10%, an arbitrary standard set by epidemiologists and gynecological oncologists[38]. Screening using CA 125 alone is neither sensitive nor specific enough. Significant expression of CA125 was observed in 80% of ovarian cancers at the tissue level[39], indicating the sensitivity of a CA125-based screening assay should not exceed 80%. In addition, CA125 is not cancer specific. In post-menopausal women, CA125 exhibits a specificity of 99%, not high enough to achieve a PPV of 10%[38]. In pre-menopausal women, serum CA125 is elevated in a variety of benign conditions, including but not limited to pregnancy, endometriosis, ovarian cysts, and pelvic inflammation. CA125 can also be elevated in other types of cancer such as carcinoma of the breast and lung. Due to the lack of sensitivity and specificity, CA125 alone is not recommended to screen asymptomatic women for ovarian cancer[40].

Multiple approaches have been proposed to enhance both the sensitivity and specificity of CA125. The Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening trial evaluates concurrent combination of CA125 and ultrasound, in which if CA125 is elevated or a pelvic lesion encountered by transvaginal ultrasound (TVUS), patients are referred to their local

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

physicians for management. According to a preliminary report from this study, CA125 alone had a PPV of 3.7% for detection of ovarian cancer; TVUS had a PPV of 1%; and combined together had a PPV of 23.5%[41]. Although the evaluation requires longer follow-up, the predictive value of both tests was relatively low.

A sequential combination of CA125 and ultrasound may be more effective. Two major trials have been conducted in the United Kingdom using a sequential combination. One studied postmenopausal women older than 45 years. If CA125 levels were greater than 30 U/mL, TVUS was performed; if TVUS results were abnormal, surgery was undertaken[42]. Among 10,985 women screened, the median survival in the screened group (73 months) was significantly greater ($p = 0.012$) than in the control group[42], although this may be due to the lead-time bias of screening. A more recent study using the sequential combination also reported encouraging results: the sensitivity, specificity, and PPV for all primary ovarian and tubal cancers were 89.4%, 99.8%, and 43.3%, respectively[43]. The effect of the screening on mortality remains to be determined.

The risk of ovarian cancer algorithm (ROCA) using serial CA125 levels to improve the specificity is based on two observations: (i) patients with benign diseases tend to have stable levels of CA125, even when they are elevated and (ii) patients with ovarian cancer generally have progressively rising values. ROCA has proved superior to a fixed cutoff for identifying women at increased risks of ovarian cancer: when the specificity was set at 98%, ROCA achieved a sensitivity of 86%, a significant improvement over the sensitivity of 62% using the arbitrary cutoff of 30 U/mL[38].

4. AFP

AFP is a 70kD glycoprotein with 591 amino acids and 4% carbohydrate by weight. During fetal life, AFP is first produced by the yolk sac and later by the fetal liver, resulting in a very high plasma level. After birth, AFP levels decrease, reaching adult levels of less than 10 ng/mL at 12–18 months.

AFP is not specific for hepatocellular carcinoma (HCC). Elevated serum AFP levels occur not only in HCC, but also in pregnancy, hepatitis and liver cirrhosis, and other malignancies such as tumors of gonadal origin and the gastrointestinal tract[44,45]. AFP is not sensitive for HCC either since not all HCC tumors secrete AFP. AFP levels are normal in up to 40 percent of small HCCs at the tissue level and in the majority of patients with fibrolamellar carcinoma, a variant of HCC[46,47].

Nevertheless, screening for HCC with AFP in combination with liver ultrasound is widely practiced for two reasons[48,49]. First, surgical resection and liver transplantation are the only options for long-term survival in people with HCC. Both procedures have limited efficacy once symptoms develop. Second, carriers of the hepatitis B and C viruses are at a very high risk of developing HCC. Therefore, screening asymptomatic subjects with hepatitis may lead to early and effective treatment.

Depending upon the individual immunoassay, the upper limits of the reference range of AFP are set between 10 and 25 ng/mL. Modest elevations of serum AFP between 10 and 500 ng/mL occur in adult patients with hepatitis and liver cirrhosis, representing a diagnostic gray zone of AFP. It is generally accepted that AFP levels greater than 500 ng/L in high-risk patients are diagnostic of HCC. However, a lower AFP level is used for screening. Using a cut off of 20 ng/mL, the sensitivity of AFP ranges from 41–65% with specificity from 80–94% in multiple studies [50]. In order to increase specificity, AFP is used in combination with liver ultrasound. Multiple studies have shown that screening for HCC using AFP and liver ultrasound

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

could identify tumors at an early stage[51–53]. However, the clinical benefits of early detection on mortality remain to be determined.

AFP has multiple glycoforms. Chan *et al* demonstrated AFP could be separated into the "yolk-sac type" and the "liver type" by affinity chromatography on *concanavalin A* (Con-A) Sepharose[54]. In another study, Sato *et al* showed that AFP from benign chronic liver diseases and HCC bound differentially to lectin *Lens culinaris agglutinin* (LCA)[55]. AFP is fractionated into 3 glycoforms L1, L2, and L3 based on their reactivity with LCA. The L3 fraction binds strongly to LCA[56]. Imaging studies showed that AFP-L3 positive HCC was hypervascular with blood supplies derived from hepatic artery, and had a shorter doubling time [57]. Therefore, AFP-L3 may identify a more aggressive form of HCC.

An AFP-L3 immunoassay is commercially available and has been widely adopted in Japan. This assay is based on liquid phase binding of the AFP-L3 glycoform with LCA and two specific antibodies labeled with peroxidase and polysulfated tyrosine peptide, respectively [58]. A cut off of 10% AFP-L3, calculated using the ratio of AFP-L3 to total AFP, is used to identify patients with risks of small tumors < 2cm in diameter, arguably defined as aggressive cancer in clinical settings[57,59]. A recent case-control study conducted in 7 academic medical centers in the United States showed that AFP-L3 was not useful for the early detection of HCC [60]. This study supported by the Early Detection Research Network (EDRN) of the United States National Cancer Institute (NCI) compared performance of AFP, des-gamma carboxyprothrombin (DCP) and AFP-L3 for the diagnosis of early HCC. A total of 836 patients were enrolled; 417 (50%) were cirrhosis controls and 419 (50%) were HCC cases, of which 208 (49.6%) had early stage HCC (n=77 very early, n=131 early). Results showed AFP was more sensitive than DCP and AFP-L3 for the diagnosis of early and very early stage HCC. At a new cutoff of 10.9 ng/mL, AFP had a sensitivity of 66% and specificity of 81%. AFP-L3 was not useful for the diagnosis of early stage HCC, likely due to the need for an elevated total AFP.

5. Future of Early Detection of Cancer

Since cancer is not a single disease but a composite of multiple diseases, it is likely that a successful strategy for early detection using plasma tumor markers will require a panel of biomarkers instead of a single biomarker as has been pursued in the past. These biomarker panels could consist of not only tumor antigens but also antibodies against tumor antigens. Immune responses directed against tumor antigens are a promising approach for biomarker discovery [61]. The immune system responds to cancer cells in two ways, by reacting against tumor-specific antigens (molecules that are unique to cancer cells) or against tumor-associated antigens (molecules that are expressed differently by cancer cells and normal cells)[62]. Evidence of these responses has been demonstrated by identification of autoantibodies for tumor antigens from patients with different cancer types, also known as autoantibody profiling or immunoproteomics.

Immunoproteomics uses proteomic technologies such as protein microarray and mass spectrometry. Recombinant protein microarrays containing over 5000 human proteins were used to screen for autoantibodies using serum from 30 patients with ovarian cancer and 30 healthy individuals. Ninety four antigens were identified that had higher reactivity with cancer sera than normal[63]. One concern of using recombinant proteins is that immunogenicity may depend on post-translational modifications and other types of protein processing that may be tumor specific[64]. Therefore, microarrays containing a repertoire of natural proteins isolated from tumors or tumor-derived cell lines may be best to determine immunogenicity. The other common approach to immunoproteomics is mass spectrometry. A mass-spectrometry-based

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

method has been developed to identify autoantibody-based serum biomarkers for the early diagnosis of ovarian cancer[65].

Whether antigens or autoantibodies are used, a multiple marker strategy combines the merits of single markers and could result in both improved sensitivity and specificity over a single marker. Unfortunately, most early studies using multiple markers have improved sensitivity at the expense of a marked decrease in specificity. Recently, using appropriate statistical or bioinformatic methods, multiple marker strategies have improved sensitivity while maintaining specificity. One study by Zhang *et al.* showed the combination of four serum markers CA125II, CA72-4, CA15-3, and macrophage colony stimulating factor (M-CSF) through an Artificial Neural Network (ANN) model improved the overall accuracy to discern healthy women from patients with early stage ovarian cancer. At a fixed specificity of 98%, the sensitivities for ANN and CA125II alone were 71% (37/52) and 46% (24/52) (p=0.047), respectively, for detecting early stage epithelial ovarian cancer, and 71% (30/42) and 43% (18/42) (p=0.040), respectively, for detecting invasive early stage epithelial ovarian cancer [66]. In another study to improve the detection of early stage ovarian cancer, three proteomic biomarkers were identified as apolipoprotein A1 (down-regulated in cancer), a truncated form of transthyretin (down-regulated), and a cleavage fragment of inter-alpha-trypsin inhibitor heavy chain H4 (up-regulated). The sensitivity of a multivariate model combining the three biomarkers and CA125 was 74%, higher than that of CA125 alone of 65% at a matched specificity of 97%. When compared at a fixed sensitivity of 83%, the specificity of the model was significantly better than that of CA125 alone (94% versus 52%)[67].

6. Conclusion

Despite issues with sensitivity and/or specificity, PSA, CA125, and AFP have been used clinically for the early detection of prostate, ovarian, and liver cancer, respectively. Many strategies have been used to improve the sensitivity or specificity of these markers, including calculation of their changes over time, measurement of subfractions of these markers that are more cancer-specific, and combinations with other markers or imaging modalities. Immunoassays for these plasma tumor markers are commercially available. The results from these assays, however, are not interchangeable due to two fundamental principles of immunoassays: metrological traceability and antibody specificity. Therefore, standardization of these immunoassays will help to make the results more comparable. Identification of autoantibodies to tumor antigens and combinations of independent plasma tumor antigens are two promising future directions for the early detection of cancer.

7. Expert Opinion

Immunoassay of plasma tumor markers is important for two reasons. First, for the markers that have established clinical utility, immunoassays provide quantitative analysis of these markers in plasma and thus provide clinicians information for making medical decisions. Second, for candidate markers that need further validation, development of immunoassays is essential for establishing clinical performance of these markers. In fact, one limiting factor for many validation approaches is the lack of well-characterized, high-quality antibodies. . Realizing this obstacle, an emerging partnership has been developed between the public and private sectors for development of high quality antibodies toward human proteins. Examples are the Human Antibody Initiative by the Human Proteome Organization (HUPO) and the Clinical Proteomic Technologies for Cancer program (CPTAC) by the U.S. National Cancer Institute (NCI). These noteworthy efforts will hopefully speed up the validation process and lead to more immunoassays that are potentially useful for early detection of cancer.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Finally, increased understanding of cancer biology could improve the screening strategies for cancer. It is still controversial whether tumor markers released in the earliest stage of cancer can be detected in serum with sufficient sensitivity and specificity. An understanding of the rate of protein release from tumors at early stages and the extent of release of the same proteins from surrounding cell populations will, therefore, be helpful to answer this question. In the end, the development of plasma tumor associated biomarkers for early detection is an area of cancer research that presents both challenges and opportunities. Currently, the majority of protein biomarkers discovered using clinical proteomics for cancer have failed to show relevant clinical utility in subsequent validation studies due to issues such as the complexity of serum proteome, the biological variability of protein expressions in serum, the variability in sample processing and handling, and the analytical variability[68–70]. Realizing these challenges, it is critical to incorporate biological, clinical, and epidemiologic knowledge of cancer into the entire process of cancer biomarker discovery from study design, analytical processing, to data analysis[69,71]. The success of this venture will require a team of dedicated scientists with expertise in cancer biology, oncology, statistics and pathology, such as the Early Detection Research Network (EDRN) of the US National Cancer Institute.

Bibliography

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. CA Cancer J Clin 2008 Mar–Apr;58(2):71–96. [PubMed: 18287387]
2. Etzioni R, Urban N, Ramsey S, et al. The case for early detection. Nat Rev Cancer 2003 Apr;3(4):243–252. [PubMed: 12671663]
3. Smith RA, Cokkinides V, Eyre HJ. American Cancer Society guidelines for the early detection of cancer, 2006. CA Cancer J Clin 2006 Jan–Feb;56(1):11–25. quiz 49–50. [PubMed: 16449183]
4. Sokoll LJ, Chan DW. Prostate-specific antigen. Its discovery and biochemical characteristics. The Urologic clinics of North America 1997 May;24(2):253–259. [PubMed: 9126221]
5. Brawer MK. Prostate-specific antigen: current status. CA Cancer J Clin 1999 Sep–Oct;49(5):264–281. [PubMed: 11198954]
6. Lilja H, Haese A, Bjork T, et al. Significance and metabolism of complexed and noncomplexed prostate specific antigen forms, and human glandular kallikrein 2 in clinically localized prostate cancer before and after radical prostatectomy. J Urol 1999 Dec;162(6):2029–2034. discussion 34–35. [PubMed: 10569562]
7. Meany DL, Chan DW. Comparability of tumor marker immunoassays: still an important issue for clinical diagnostics? Clin Chem Lab Med 2008;46(5):575–576. [PubMed: 18598200]
8. Belanger A, van Halbeek H, Graves HC, et al. Molecular mass and carbohydrate structure of prostate specific antigen: studies for establishment of an international PSA standard. The Prostate 1995 Oct; 27(4):187–197. [PubMed: 7479385]
9. Stamey TA, Teplow DB, Graves HC. Identity of PSA purified from seminal fluid by different methods: comparison by amino acid analysis and assigned extinction coefficients. The Prostate 1995 Oct;27(4): 198–203. [PubMed: 7479386]
10. Stephan C, Kahrs AM, Klotzek S, et al. Toward metrological traceability in the determination of prostate-specific antigen (PSA): calibrating Beckman Coulter Hybritech Access PSA assays to WHO standards compared with the traditional Hybritech standards. Clin Chem Lab Med 2008;46(5):623–629. [PubMed: 18839463]
11. Chan DW, Sokoll LJ. Prostate-specific antigen: update 1997. Journal of the International Federation of Clinical Chemistry / IFCC 1997 Sep;9(3):120–125. [PubMed: 10174623]
12. Sokoll LJ, Witte DL, Klee GG, et al. Redesigned proficiency testing materials improve survey outcomes for prostate-specific antigen. A College of American Pathologists Ligand Assay Survey tool. Archives of pathology & laboratory medicine 2000 Nov;124(11):1608–1613. [PubMed: 11079010]
13. Schroder FH, Hugosson J, Roobol MJ, et al. Screening and prostate-cancer mortality in a randomized European study. N Engl J Med 2009 Mar 26;360(13):1320–1328. [PubMed: 19297566]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

14. Andriole GL, Crawford ED, Grubb RL 3rd, et al. Mortality results from a randomized prostate-cancer screening trial. N Engl J Med 2009 Mar 26;360(13):1310–1319. [PubMed: 19297565]
15. Barry MJ. Screening for prostate cancer--the controversy that refuses to die. N Engl J Med 2009 Mar 26;360(13):1351–1354. [PubMed: 19297564]
16. Thompson IM, Pauler DK, Goodman PJ, et al. Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter. N Engl J Med 2004 May 27;350(22):2239–2246. [PubMed: 15163773]
17. Sturgeon CM, Duffy MJ, Stenman UH, 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 Dec;54(12):e11–e79. [PubMed: 19042984]
18. Catalona WJ, Partin AW, Slawin KM, et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. Jama 1998 May 20;279(19):1542–1547. [PubMed: 9605898]
19. Woodrum D, French C, Shamel LB. Stability of free prostate-specific antigen in serum samples under a variety of sample collection and sample storage conditions. Urology 1996 Dec;48:33–39. [PubMed: 8973697]
20. Paus E, Nilsson O, Bormer OP, et al. Stability of free and total prostate specific antigen in serum from patients with prostate carcinoma and benign hyperplasia. J Urol 1998 May;159(5):1599–1605. [PubMed: 9554362]
21. Haese A, Graefen M, Noldus J, et al. Prostatic volume and ratio of free-to-total prostate specific antigen in patients with prostatic cancer or benign prostatic hyperplasia. J Urol 1997 Dec;158(6):2188–2192. [PubMed: 9366341]
22. Partin AW, Catalona WJ, Southwick PC, et al. Analysis of percent free prostate-specific antigen (PSA) for prostate cancer detection: influence of total PSA, prostate volume, and age. Urology 1996 Dec;48:55–61. [PubMed: 8973701]
23. Loeb S, Kettermann A, Ferrucci L, et al. PSA doubling time versus PSA velocity to predict high-risk prostate cancer: data from the Baltimore Longitudinal Study of Aging. Eur Urol 2008 Nov;54(5):1073–1080. [PubMed: 18614274]
24. Carter HB, Pearson JD, Metter EJ, et al. Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease. Jama 1992 Apr 22–29;267(16):2215–2220. [PubMed: 1372942]
25. Carter HB, Pearson JD. PSA velocity for the diagnosis of early prostate cancer. A new concept. The Urologic clinics of North America 1993 Nov;20(4):665–670. [PubMed: 7505974]
26. Carter HB, Ferrucci L, Kettermann A, et al. Detection of life-threatening prostate cancer with prostate-specific antigen velocity during a window of curability. J Natl Cancer Inst 2006 Nov 1;98(21):1521–1527. [PubMed: 17077354]
27. Carter HB, Kettermann A, Ferrucci L, et al. Prostate-specific antigen velocity risk count assessment: a new concept for detection of life-threatening prostate cancer during window of curability. Urology 2007 Oct;70(4):685–690. [PubMed: 17991538]
28. Loeb S. Prostate cancer: Is PSA velocity useful? Nat Rev Urol 2009 Jun;6(6):305–306. [PubMed: 19498408]
29. Carter HB, Partin AW, Luderer AA, et al. Percentage of free prostate-specific antigen in sera predicts aggressiveness of prostate cancer a decade before diagnosis. Urology 1997 Mar;49(3):379–384. [PubMed: 9123702]
30. Partin AW, Carter HB, Chan DW, et al. Prostate specific antigen in the staging of localized prostate cancer: influence of tumor differentiation, tumor volume and benign hyperplasia. J Urol 1990 Apr;143(4):747–752. [PubMed: 1690309]
31. Oesterling JE. Age-specific reference ranges for serum PSA. N Engl J Med 1996 Aug 1;335(5):345–346. [PubMed: 8663860]
32. Reissigl A, Pointner J, Horninger W, et al. Comparison of different prostate-specific antigen cutpoints for early detection of prostate cancer: results of a large screening study. Urology 1995 Nov;46(5):662–665. [PubMed: 7495117]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

33. Littrup PJ, Kane RA, Mettlin CJ, et al. Cost-effective prostate cancer detection. Reduction of low-yield biopsies. Investigators of the American Cancer Society National Prostate Cancer Detection Project. Cancer 1994 Dec 15;74(12):3146–3158. [PubMed: 7526969]
34. Partin AW, Brawer MK, Bartsch G, et al. Complexed prostate specific antigen improves specificity for prostate cancer detection: results of a prospective multicenter clinical trial. J Urol 2003 Nov;170 (5):1787–1791. [PubMed: 14532777]
35. Sokoll LJ, Wang Y, Feng Z, et al. [−2]proenzyme prostate specific antigen for prostate cancer detection: a national cancer institute early detection research network validation study. J Urol 2008 Aug;180(2):539–543. discussion 43. [PubMed: 18550118]
36. Yin BW, Lloyd KO. Molecular cloning of the CA125 ovarian cancer antigen: identification as a new mucin, MUC16. The Journal of biological chemistry 2001 Jul 20;276(29):27371–27375. [PubMed: 11369781]
37. Nustad K, Bast RC Jr. Brien TJ, et al. Specificity and affinity of 26 monoclonal antibodies against the CA 125 antigen: first report from the ISOBM TD-1 workshop. International Society for Oncodevelopmental Biology and Medicine. Tumour Biol 1996;17(4):196–219. [PubMed: 8685601]
38. Badgwell D, Bast RC Jr. Early detection of ovarian cancer. Dis Markers 2007;23(5–6):397–410. [PubMed: 18057523]
39. Rosen DG, Wang L, Atkinson JN, et al. Potential markers that complement expression of CA125 in epithelial ovarian cancer. Gynecol Oncol 2005 Nov;99(2):267–277. [PubMed: 16061277]
40. Duffy MJ, Bonfrer JM, Kulpa J, et al. CA125 in ovarian cancer: European Group on Tumor Markers guidelines for clinical use. Int J Gynecol Cancer 2005 Sep–Oct;15(5):679–691. [PubMed: 16174214]
41. Buys SS, Partridge E, Greene MH, et al. Ovarian cancer screening in the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial: findings from the initial screen of a randomized trial. American journal of obstetrics and gynecology 2005 Nov;193(5):1630–1639. [PubMed: 16260202]
42. Jacobs IJ, Skates SJ, MacDonald N, et al. Screening for ovarian cancer: a pilot randomised controlled trial. Lancet 1999 Apr 10;353(9160):1207–1210. [PubMed: 10217079]
43. Menon U, Gentry-Maharaj A, Hallett R, et al. Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer, and stage distribution of detected cancers: results of the prevalence screen of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). Lancet Oncol 2009 Apr;10(4):327–340. [PubMed: 19282241]
44. Masopust J, Kithier K, Radl J, et al. Occurrence of fetoprotein in patients with neoplasms and non-neoplastic diseases. International journal of cancer 1968 May 15;3(3):364–373.
45. McIntire KR, Waldmann TA, Moertel CG, et al. Serum alpha-fetoprotein in patients with neoplasms of the gastrointestinal tract. Cancer research 1975 Apr;35(4):991–996. [PubMed: 46783]
46. Chen DS, Sung JL, Sheu JC, et al. Serum alpha-fetoprotein in the early stage of human hepatocellular carcinoma. Gastroenterology 1984 Jun;86(6):1404–1409. [PubMed: 6201411]
47. Soreide O, Czerniak A, Bradpiece H, et al. Characteristics of fibrolamellar hepatocellular carcinoma. A study of nine cases and a review of the literature. American journal of surgery 1986 Apr;151(4): 518–523. [PubMed: 2421594]
48. McMahon BJ, Bulkow L, Harpster A, et al. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study. Hepatology (Baltimore, Md 2000 Oct;32(4 Pt 1):842–846.
49. Colombo M. Screening for cancer in viral hepatitis. Clinics in liver disease 2001 Feb;5(1):109–122. [PubMed: 11218910]
50. Marrero JA. Screening tests for hepatocellular carcinoma. Clinics in liver disease 2005 May;9(2): 235–251. vi. [PubMed: 15831271]
51. Yuen MF, Cheng CC, Lauder IJ, et al. Early detection of hepatocellular carcinoma increases the chance of treatment: Hong Kong experience. Hepatology (Baltimore, Md 2000 Feb;31(2):330–335.
52. Tong MJ, Blatt LM, Kao VW. Surveillance for hepatocellular carcinoma in patients with chronic viral hepatitis in the United States of America. Journal of gastroenterology and hepatology 2001 May;16(5):553–559. [PubMed: 11350553]
53. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. Journal of cancer research and clinical oncology 2004 Jul;130(7):417–422. [PubMed: 15042359]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

54. Chan DW, Miao YC. Affinity chromatographic separation of alpha-fetoprotein variants: development of a mini-column procedure, and application to cancer patients. Clin Chem 1986 Dec;32(12):2143–2146. [PubMed: 2430738]
55. Sato Y, Nakata K, Kato Y, et al. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. N Engl J Med 1993 Jun 24;328(25):1802–1806. [PubMed: 7684823]
56. Li D, Mallory T, Satomura S. AFP-L3: a new generation of tumor marker for hepatocellular carcinoma. Clinica chimica acta; international journal of clinical chemistry 2001 Nov;313(1–2):15–19. [PubMed: 11694234]
57. Kumada T, Nakano S, Takeda I, et al. Clinical utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in small hepatocellular carcinoma: special reference to imaging diagnosis. Journal of hepatology 1999 Jan;30(1):125–130. [PubMed: 9927159]
58. Katoh H, Nakamura K, Tanaka T, et al. Automatic and simultaneous analysis of Lens culinaris agglutinin-reactive alpha-fetoprotein ratio and total alpha-fetoprotein concentration. Analytical chemistry 1998 May 15;70(10):2110–2114. [PubMed: 9608849]
59. Yamashiki N, Seki T, Wakabayashi M, et al. Usefulness of Lens culinaris agglutinin A-reactive fraction of alpha-fetoprotein (AFP-L3) as a marker of distant metastasis from hepatocellular carcinoma. Oncology reports 1999 Nov–Dec;6(6):1229–1232. [PubMed: 10523686]
60. Marrero JA, Feng Z, Wang Y, et al. Alpha-fetoprotein, Des-gamma Carboxyprothrombin, and Lectin-Bound Alpha-fetoprotein in Early Hepatocellular Carcinoma AFP, DCP, and AFP-L3 in Hepatocellular carcinoma. Gastroenterology 2009 Apr;:8. [PubMed: 19161632]
61. Hanash SM, Pitteri SJ, Faca VM. Mining the plasma proteome for cancer biomarkers. Nature 2008 Apr 3;452(7187):571–579. [PubMed: 18385731]
62. Finn OJ. Cancer immunology. N Engl J Med 2008 Jun 19;358(25):2704–2715. [PubMed: 18565863]
63. Hudson ME, Pozdnyakova I, Haines K, et al. Identification of differentially expressed proteins in ovarian cancer using high-density protein microarrays. Proceedings of the National Academy of Sciences of the United States of America 2007 Oct 30;104(44):17494–17499. [PubMed: 17954908]
64. Madoz-Gurpide J, Kuick R, Wang H, et al. Integral protein microarrays for the identification of lung cancer antigens in sera that induce a humoral immune response. Mol Cell Proteomics 2008 Feb;7(2):268–281. [PubMed: 17916591]
65. Philip R, Murthy S, Krakover J, et al. Shared immunoproteome for ovarian cancer diagnostics and immunotherapy: potential theranostic approach to cancer. Journal of proteome research 2007 Jul;6(7):2509–2517. [PubMed: 17547437]
66. Zhang Y, Zhang Y, Adachi J, et al. MAPU: Max-Planck Unified database of organellar, cellular, tissue and body fluid proteomes. Nucleic Acids Res 2007 Jan;35(Database issue):D771–D779. [PubMed: 17090601]
67. Zhang Z, Bast RC Jr. Yu Y, et al. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. Cancer research 2004 Aug 15;64(16):5882–5890. [PubMed: 15313933]
68. Rai AJ, Gelfand CA, Haywood BC, et al. HUPO Plasma Proteome Project specimen collection and handling: towards the standardization of parameters for plasma proteome samples. Proteomics 2005 Aug;5(13):3262–3277. [PubMed: 16052621]
69. Zhang Z, Chan DW. Cancer proteomics: in pursuit of "true" biomarker discovery. Cancer Epidemiol Biomarkers Prev 2005 Oct;14(10):2283–2286. [PubMed: 16214905]
70. Zhang H, Chan DW. Cancer biomarker discovery in plasma using a tissue-targeted proteomic approach. Cancer Epidemiol Biomarkers Prev 2007 Oct;16(10):1915–1917. [PubMed: 17932335]
71. Plasencia C, Dayam R, Wang Q, et al. Discovery and preclinical evaluation of a novel class of small-molecule compounds in hormone-dependent and -independent cancer cell lines. Mol Cancer Ther 2005 Jul;4(7):1105–1113. [PubMed: 16020668]