Review

**Serological Autoantibodies as Diagnostic Cancer Biomarkers – a review**

Pauline Zaenker¹, Melanie R. Ziman¹,²

¹School of Medical Sciences, Edith Cowan University, Perth, WA, Australia

²Department of Pathology and Laboratory Medicine, The University of Western Australia, Crawley, Australia

**Correspondence**
Professor Melanie Ziman
Director ECU Melanoma Research Foundation
Edith Cowan University (ECU)
270 Joondalup Drive, Joondalup, Perth, WA 6027
Ph: +61 - 8 – 63043640
Fax: +61 -8– 63042626
Mob: +61 (0) 419929851
E-mail: m.ziman@ecu.edu.au

**Funding sources**
NHMRC application numbers 1046711 and 1013349
Edith Cowan University strategic funds

**Conflict of interest**
None declared

**Submission declaration**
We confirm that this review has not been published elsewhere and is not under consideration by another journal. All authors have approved the review and agree with the submission to the Journal of Autoimmunity.

**Abbreviations**

PAMPs, pathogen-associated molecular patterns; DAMPs, danger-associated molecular patterns; PRRs, soluble pattern recognition receptors; TAAs, tumour-associated antigens; TLR4, toll-like receptor 4; PTMs, post-translational modifications; MHC, major histocompatibility complex(es); PCAA, prostate-cancer-associated antigen; RA, Rheumatoid arthritis; SLE, Systemic lupus erythematosus; PRMS, primary progressive Multiple sclerosis; MS, Multiple sclerosis; CRC, Colorectal cancer; SEREX, serological analysis of tumour antigens by recombinant cDNA expression cloning; SERPA, serological proteome analysis; DCIS, Ductal Carcinoma in Situ; ELISA, enzyme-linked immunosorbent assay; HCC, Hepatocellular carcinoma; HBV, Chronic hepatitis B virus; mTOR, rapamycin-sensitive mammalian target of rapamycin; 2D-PAGE, two dimensional polyacrylamide gel electrophoresis

**Abstract**

Current diagnostic techniques utilised for the early detection of cancers are successful but subject to detection bias. A recent focus lies in the development of more accurate diagnostic
tools. A rise in serological autoantibody levels has been shown to precede the development of cancer disease symptoms. Therefore, autoantibody levels in patient blood serum have been proposed as diagnostic biomarkers for early-stage diagnosis of cancers. Their clinical application has however, been hindered by low sensitivity, specificity and low predictive value scores. These scores have been shown to improve when panels of multiple diagnostic autoantibody biomarkers are utilised. A five marker biomarker panel has been shown to increase the sensitivity of prostate cancer diagnosis to 95% compared to 12.2% for prostate-specific antigen alone. New potential biomarker panels were also discovered for lung, colon and stomach cancer diagnosis with sensitivity of 76%, 65.4% and 50.8% respectively. Studies in breast and liver cancer however, seem to favour single markers, namely alpha-2-HS-glycoprotein and des-gamma-carboxyprothrombin with sensitivities of 79% and 89% for the early-detection of the cancers. The aim of this review is to discuss the relevance of autoantibodies in cancer diagnosis and to outline the current methodologies used in the detection of autoantibodies. The review concludes with a discussion of the autoantibodies currently utilised in the diagnosis of cancers of the prostate, breast, lung, colon, stomach and liver. A discussion of the potential future use of autoantibodies as diagnostic cancer biomarkers is also included in this review.

**Keywords**

Autoantibody, cancer, diagnosis, biomarker, autoimmunity, methodology

1) Introduction

Worldwide, cancer is the second leading cause of death (1, 2). Despite tremendous efforts to develop strategies against cancer-related mortality, the battle with high cancer mortality rates continues (3, 4). To counteract these mortality rates, research has focused on the development of diagnostic tools that enable the diagnosis of a cancer earlier before it progresses to an often
incurable metastatic stage (5). Autoantibody levels in patient blood serum have been proposed as diagnostic biomarkers for early stage diagnosis of cancers, since an increase in serum levels of certain autoantibodies has been shown to precede the development of disease symptoms (6, 7) and correlate with cancer incidence (8) for cancers of the breast (9), lung and small-cell lung (10, 11), colon (12), ovary (13) prostate (14) and head and neck cancer (15, 16).

Theories of the process of autoantibody production in cancer are complex and not yet fully understood. The immune response towards tumour-associated antigens (TAAs) presented in early stages of carcinogenesis, is thought to occur in response to cancer immunosurveillance, the process by which the immune system recognizes and destroys invading pathogens as well as host cells that have become cancerous (17-19). It has also been suggested that genetic, hormonal and environmental influences may play a part in triggering autoimmunity.

Immunologic processes causing autoantibody production are believed to be generated by the immune system in response to mutations, degradation, over-expression of proteins and/or the release of proteins from damaged tissue (20-23). Autoantibody production is also believed to be caused by mis-presentation or mis-folding of proteins which may be recognized by the immune system leading to autoantibody production and therefore, tumour-associated antigens (TAAs) or proteins that have undergone alternate post-translational modifications (PTMs) may be recognised as non-autologous (17, 19, 24), i.e.: their phosphorylation, glycosylation, oxidation or proteolytic cleavage could generate a neo-epitope or enhance self-epitope presentation and affinity to the major histocompatibility complex or T-cell receptor, inducing an immune response (25). A neo-epitope is an epitope which is located within an unexposed region of the protein, preventing any interaction between the molecule and antibodies or lymphocytes, therefore avoiding the induction of an immune response against the molecule.
The neo-epitope may only cause an immune response or tolerance when it’s structure is exposed by a conformational change or stereochemical alteration of the protein structure (26).

Here we discuss the relevance of autoantibodies in cancer diagnosis, autoantibody production in response to cancers, current methodologies utilised in the detection of autoantibodies, currently utilised autoantibodies in the diagnosis of cancers of the prostate, breast, lung, colon, stomach and liver as well as the potential future use of autoantibodies as diagnostic cancer biomarkers. A comprehensive search of electronic databases such as PubMed, NIH, UWA library and ECU library and others was performed from November 2012 to August 2013. This review included studies which were published within the last 10 years from 2003 to 2013 that reported on “currently utilised autoantibody detection methods”, “serological diagnostic cancer biomarkers” and “diagnostic autoantibody cancer biomarkers”.

2) Diagnostic relevance of autoantibodies as biomarkers in cancer

Currently, the diagnosis of the majority of cancers is restricted to the examination of the patient’s primary tumour by morphological and immunohistochemical analysis. More recently, the use of autoantibodies towards autologous tumour-associated antigens (TAAs) has been gathering momentum as these have been detected in the asymptomatic stage of cancer and may therefore serve as diagnostic biomarkers (27-31). In fact, autoantibodies have been found to precede the manifestation of clinical signs of tumour progression by several months to years (17, 32-34). One example of the potential of serological autoantibodies to diagnose early-stage cancer is the discovery of the ECPKA autoantibody as a universal cancer biomarker. In healthy mammalian cells, cAMP-dependent protein kinase A (PKA), is an intracellular enzyme. In most cancers, including those forming the subject of this review, this enzyme is secreted into the circulatory system. Once secreted, the protein is known as extracellular protein kinase A (ECPKA). This antibody was found to be elevated in a wide
range of cancers of various stages of malignancies in different cell types including bladder, breast, cervical, colon, esophageal, gastric, liver, lung, ovarian, prostate, pancreatic, renal, small bowel, rectal, adenocystic carcinomas, melanoma, sarcoma, thyoma, liposarcoma and leiomyosarcoma compared to healthy controls. Blood ECPKA levels are increased and ECPKA levels decreased after surgical removal of solid tumours (35). With the assumption that this excretion results in the production of anti-ECPKA antibodies, an enzyme immunoassay measuring the IgG of this autoantibody was developed and the sensitivity and specificity of this biomarker for detecting the incidence of 20 different cancers was calculated to be 90% and 87%. Anti-ECPKA autoantibody was detected in 90% of the patient samples and in only 13% of the control samples, indicating that the presence of the ECPKA autoantibody in sera correlates with cancer incidence (8). Furthermore, autoantibodies are easily extracted from blood serum and are generally stable and bind with high specificity to their specific antigenic proteins (36).

To date, no single autoantibody biomarker has been utilised as a cancer biomarker due to the low sensitivity and specificity of single markers. Panels of multiple tumour-associated autoantibodies with high specificity and sensitivity are sought therefore for translation into simple biomarker panel tests for routine clinical diagnosis of early-stage cancer. (17, 19, 37-40).

3) Methodology of autoantibody detection

To advance the discovery of novel combinations of autoantibody biomarkers, techniques which allow the simultaneous screening of multiple biomarkers are required. Examples of such methodologies include serological analysis of tumour antigens by recombinant cDNA expression cloning (SEREX), phage display, serological proteome analysis (SERPA), multiple affinity protein profiling (MAPPing) or protein microarrays. Please refer to figure 1.
and table 1 for a comprehensive overview and comparison of methodologies and associated processes used to detect multiple autoantibodies simultaneously.

**Serological analysis of tumour antigens by recombinant cDNA expression cloning (SEREX)**

SEREX, the serological analysis of tumour antigens by recombinant cDNA expression cloning, was first developed in 1995 (41, 42). This technique utilises antibody reactivity with autologous cancer patient sera to identify immunogenic tumour proteins (17, 39). The cDNA expression library utilised in this methodology is constructed from tumour specimens of interest and then cloned into λ-phage expression vectors which are used to transfect *Escherichia coli*. The resulting recombinant proteins are then transferred onto a nitrocellulose membrane which is incubated with diluted patient sera. Clones which are reactive with high-titer IgG antibodies are identified using an enzyme conjugated secondary antibody specific for human IgG. The cDNA clone is sequenced and the autoantigen identified. The major advantage of using SEREX is the fact that it allows the identification of tumour-associated antigens from *in vivo* material. Another advantage of this technology is that it allows the identification of several tumour-specific antigens in one experiment. Furthermore, both the tumour-specific antigen and it’s coding cDNA are present in the same plaque when immunoscreening is performed which allows the subsequent sequencing of matched cDNA immediately. The disadvantage of SEREX is the high likelihood of false-positive results. Secondly, the use of tumour tissue from a single cancer patient followed by screening with autologous patient sera limits identification of tumour-associated antigens to that patient. Moreover, this complex methodology does not detect alternate tumour-associated post-translational modifications of antigens (17). Patients may also exhibit autoimmunity to autologous proteins and therefore irrelevant non-cancer-associated proteins may be detected. Furthermore, parallel analysis with healthy donor sera as controls cannot be performed easily.
**Phage display**

Alternatively, a cDNA phage display library is constructed directly from tumour tissue or a cancer cell line derived from patient tumour material (43). Phage clones which bind to cancer sera are identified through a differential biopanning approach (44). Alternatively, a more cost-effective method is to construct the cDNA phage display library by expressing the phage proteins fused to the antigens on the surface of bacteriophages. The phage display method has the advantage of allowing the simultaneous screening of a large number of antigens against the sera of cancer patients relative to serum of healthy individuals (14, 43). The phage-display method has a higher throughput value than the SEREX method but again, antigens with alternate post-translational modifications cannot be detected using the phage-display method (19, 45).

**Protein microarray**

The protein array methods are advantageous in that they require only minute amounts of patient sera (46) while enabling the simultaneous screening of large numbers of antigens in a single test (47-52). In this methodology, purified or recombinant as well as synthetic proteins are used. Alternatively, fractured proteins of tumour origin are spotted onto the microarray platform. Arrays are then incubated with patient and control sera (17, 19, 53, 54). The array platform can be either two dimensional (such as nitrocellulose membranes, microtitre plates or glass slides) or three dimensional (such as nanoparticles or beads). While protein microarray methods are commonly used to analyse recombinant proteins expressed from *Escherichia coli* cells, alternatively, other host expression systems such as yeast and insect cells have been used to produce libraries presenting proteins with the correct post-translational modifications. The disadvantage associated with this method is the requirement for high quality protein synthesis (55). Furthermore, studies utilising protein microarrays are time restricted due to the short shelf-life of protein arrays (19, 56).
Reverse-capture microarray

In this method, the antibodies reacting with specific proteins are spotted onto the microarray. Similar to the protein microarray, the reverse-capture microarray is incubated with tumour lysate and serum proteins and the microarrays with captured proteins are then further incubated with sera from patients and controls. The autoantibodies are detected with fluorescent-labelled secondary antibody (57-59). The advantage of the utilisation of “reverse-capture” microarray technology is the elimination of the need for recombinant proteins and allows the instant identification of cancer-specific autoantibodies. However, only known antigens and their commercially available antibodies can be analysed and immunoreactivity with post-translationally modified antigens cannot be differentiated unless antibodies which bind exclusively to these antigens are commercially available.

Serological proteome analysis (SERPA)

Serological proteome analysis (SERPA) (60) is also known as PROTEOMEX. This technique is very useful for detection of tumour-associated antigens since it incorporates an effective separation of a complex mixture of proteins based on their isoelectric points and molecular weights through 2D electrophoresis and western blotting followed by identification by mass spectrometry (19, 61, 62). Proteins from the tumour tissue of interest are transferred onto a nitrocellulose membrane and immobilised. The sera from cancer patients and controls are separately screened using the immobilised proteins. The appropriate immunoreactive profiles are compared and the cancer-associated antigenic spots are identified by mass spectrometry. Similar to the SEREX technique, the advantage of the SERPA technique is the use of in vivo derived tumour-associated antigens. Furthermore, the SERPA technique allows for the identification of tumour-specific post-translational modifications and isoforms but is limited in terms of the identification of low-abundance and transmembrane tumour-associated antigens (17, 34, 51). SERPA also enables the easy parallel analysis of tumour proteins with
healthy donor sera as controls and avoids the time-consuming construction of cDNA libraries, enabling this methodology to be completed within a few hours compared to several days for SEREX and phage-display technology. However, due to the way that western blots are prepared, SERPA can only be used to detect linear epitopes (63).

**Multiple affinity protein profiling (MAPPing)**

The MAPPing methodology incorporates 2D immunoaffinity chromatography which is followed by the identification of tumour-associated antigens by tandem mass spectrometry analysis (64). In the first phase of the initial immunoaffinity chromatography, lysate from cancer cell lines or tumour tissue containing nonspecific tumour-associated antigens is bound to IgG that was obtained from healthy controls in an immunoaffinity column. The flow-through fraction is then subjected to 2D immunoaffinity in a column that contains IgG from cancer patients and columns can be utilised in parallel (65). The tumour antigens which are captured in the patient columns are eluted and digested for identification by nano-liquid chromatography mass spectrometry. MAPPing ensures that the tumour antigens are maintained in a solution which allows the potential identification of structural epitopes. The disadvantages associated with this method include the restriction of the tumour antigen identification to antibody interactions with a low dissociation rate constant. Furthermore, immunoprecipitation using these affinity columns limits the detection of tumour antigens in more complex protein solutions, such as cell lysate.

### 4) Currently utilised diagnostic autoantibody cancer biomarkers

According to epidemiologic statistics from the Cancer Research UK (66), the most commonly diagnosed cancers worldwide include lung, breast, colorectum, stomach, prostate and liver cancers. Here we discuss currently utilised or investigated autoantibodies which may serve as
diagnostic biomarkers for the cancers mentioned above. Please refer to table 2 to see a
detailed summary of the major studies described in this review including information such as
sample size, methods utilised, protein abbreviations, full names, encoding genes, alternative
protein names and their associated cancer type as well as the accuracy of each potential
biomarker and/or biomarker panel.

4.1) Prostate cancer

The prostate-specific antigen (PSA), also known as kallikrein 3 (KLK3), is part of a family of
proteases which are known as kallikreins. These proteases are encoded by a cluster of genes
which are located within a 300 kb region on chromosome 19q13.4 (67). PSA is responsible
for the cleavage of the proteins seminogelin I and II, which leads to the liquefaction of the
semen in seminal fluid (68). PSA activity is normally confined to prostatic glandular
structures only, however disturbances of this structure such as by formation of a tumour, may
result in leakages of PSA into the circulatory system (69). The PSA blood test measures the
amount of PSA within a patient’s circulation. Any PSA level between 0-4 (ng/ml) is
considered normal, while PSA levels between 4-10 (ng/ml) are slightly elevated, PSA levels
between 10-20 (ng/ml) are moderately elevated and any PSA levels above 20 (ng/ml) are
highly elevated. A positive PSA serum level above 4 (ng/ml) concentration has diagnostic
potential in prostate cancer patients (70).

Although PSA serum levels are the most commonly utilised diagnostic test for this cancer to
date, it’s specificity is less than fifty percent, resulting in frequent false positive results (71).
The primary limitation of the use of PSA as a diagnostic biomarker is the inability to
distinguish between benign and malignant stages of the disease (72). Increased PSA serum
levels may also arise due to non-cancerous conditions such as enlargement of the prostate,
prostatitis and urinary infection (69). Xie et al. (73) developed a new multiplex assay which
they termed the “A+PSA” assay (the autoantibody +PSA assay). This assay used B cell
epitopes from previously defined prostate-cancer-associated antigen (PCAA), including New
York oesophageal squamous cell carcinoma (NY-ESO-1), synovial sarcoma X breakpoint 2,4
(SSX-2,4), X antigen family member 1B (XAGE-1b), lens epithelium-derived growth factor
(LEDGF), transferrin receptor protein 9 (p90) and alpha-methylacyl-CoA racemase
(AMACR). The platform allowed the simultaneous screening of these six autoantibodies
alongside PSA and PSA screening alone in 131 pre-surgery biopsy confirmed prostate cancer
patients and 121 prostatitis and/or benign prostatic hyperplasia patients. The overall aim of
this research was to develop a reliable platform which will enable the diagnosis of prostate
cancer patients relative to non-malignant cases. Xie et al. (73) found that PSA alone had a
sensitivity of 52% and specificity of 79% in all patients while the A+PSA platforms showed
a sensitivity of 79% and a specificity of 84% in all patients. The A+PSA platform also had a
decreased false positive outcome of only 16% versus 21% when PSA alone was utilised.
Overall, the accuracy of the A+PSA test platform was as high as 81% while PSA alone only
showed an accuracy of 65%. Wang et al., (14) utilised phage protein microarray technology
and 119 prostate cancer patient sera and 138 healthy control sera, to identify increased
autoantibody levels of bromodomain-containing protein 2 (BRD2), eukaryotic translation
initiation factor 4 gamma 1 (eIF4G1), ribosomal protein L22 (RPL22), ribosomal protein LBa
(RPL13a), and hypothetical protein XP_373908 (XP_373908) as the antigens most frequently
bound to auto-antibodies in prostate cancer patient serum. This microarray displayed 81.6%
sensitivity and 88.2% specificity. Except for hypothetical protein XP_373908, these structures
are derived from intracellular proteins involved in regulating either transcription or translation
and closely resembled autologous proteins. However, when tested, their DNA sequences were
not identical to those of genes encoding for autologous proteins (14). Moreover, the
autoantibody signature was detected in only 5 of 14 serum samples from patients who had
undergone prostatectomy and in 3 of 11 serum samples from patients with hormone-refractory disease, suggesting that the autoantibody profile is attenuated on removal of the "immunogen" or after treatment with anti-androgen chemotherapeutic agents, or both. Taken together these results provide evidence that the above mentioned autoantibodies are associated with the presence of this cancer (14). A more recent microarray study, which aimed to identify an autoantibody signature to distinguish prostate cancer from benign prostatic hyperplasia in patients who showed increased PSA levels, displayed a sensitivity of 95% and 80% specificity compared to 12.2% sensitivity and 80% specificity of PSA alone. This microarray, tested against the sera of 41 prostate cancer patients and 39 benign prostate hyperplasia patients, identified talin-1 (TLN1), TAR DNA-binding protein (TARDBP), LEDGF, Caldesmon (CALD1) and Parkinson disease (autosomal recessive, early onset) 7 oncogene (PARK7) as potential diagnostic autoantibody signature (74).

4.2) Breast cancer

Biomarkers such as carcinoma antigen 15-3 (CA 15-3), carcinoma antigen 27-29 (CA 27-29) and carcinoembryonic antigen (CEA) have been accepted for clinical use; however, due to their low sensitivity and specificity they are suggested to be utilised for the diagnosis of more advanced stages rather than for the early diagnosis of breast cancer (75). In terms of autoantibody biomarkers, antibodies to human epidermal growth factor receptor 2 (HER2) (76), tumour protein 53 (p53) (77), Mucin 1, cell surface associated (MUC1) (78) and NY-ESO-1 (79) were first discovered in breast cancer patients. In fact, antibodies to HER2/neu (76) have been detected in early stage breast cancer patients but their presence has also been detected in other cancers, limiting their use as a diagnostic biomarker for breast cancer alone (28, 30, 80). An increase to 44% sensitivity and 97.6% specificity in breast cancer detection was achieved through the successive addition of the three tumour-associated antigens p53, protein 16 (p16) and avian myelocytomatosis viral oncogene homolog (c-myc) (81). SEREX
technology was utilised by Zhong et al. (82) to detect three further breast cancer associated autoantibodies including serine active site containing 1 (SERAC1), receptor expressed in lymphoid tissues (RELT) and ankyrin repeat and suppressor of cytokine signalling (SOCS) box protein 9 (ASB-9). The combined panel of these three biomarkers achieved 77% sensitivity and 82.8% specificity when tested against 87 breast cancer patients and 87 healthy control sera (82). The SERPA approach was utilised by Desmetz et al. (83) who have identified heat shock protein 60 (HSP60) autoantibodies in a cohort consisting of 49 ductal carcinoma in situ patients, 58 early-stage breast cancer patients, 20 patients with other types of cancer, 20 patients with various autoimmune diseases and 93 healthy controls and the sensitivity of HSP60 autoantibodies as a potential biomarker for the diagnosis of breast cancer was calculated to be 31.8% while it’s specificity is 95.7%. A study by Chapman et al. (84) with a cohort of 94 healthy controls, 97 primary breast cancer sera and 40 ductal Carcinoma in Situ (DCIS) sera, tested for seven antigens including HER2, c-myc, p53, breast cancer type 1 susceptibility protein (BRCA1), breast cancer type 2 susceptibility protein (BRCA2), Ny-ESO-1 and MUC1. The specificity of the assay was found to be as high as 91-98%, even when tested for individual markers only; however, the individual autoantigen assay sensitivity was only 3-23% in the DCIS sera and 8-24% in the primary breast cancer sera. On comparison, the sensitivity increased to 45% in DCIS sera and 64% in primary cancer sera with a specificity of 85% when a combined panel of 6 out of the 7 autoantigens was tested; which, alongside other cancer detection methods such as mammography, may lead to a significant improvement in breast cancer detection. A study by Hamrita et al. (85) utilised the SERPA method to test sera from patients with more invasive breast cancer. The study found HSP60 autoantibodies in 47.5% of breast cancer patients and in only 4.7% of healthy control sera. Alpha-2-HS-glycoprotein (AHSG) autoantibodies have also been identified in 79.1% of 81 breast cancer patient samples and only in 9.6% of 73 control samples; however, the diagnostic relevance of these autoantibodies remain to be validated (80).
4.3) Lung cancer

Lung cancer is notoriously heterogeneous and therefore no diagnostic test for the early detection of this cancer has been established (86).

A study by Pereira-Faca et al. (87) used one- and two-dimensional electrophoresis as well as western blotting and mass spectrometry to identify the 14-3-3 Θ autoantibody as a potential biomarker for the early-stage diagnosis of lung cancer in a cohort consisting of 45 newly diagnosed lung cancer patients, 18 pre-diagnostic lung cancer patients and 62 matched healthy controls. This 14-3-3 Θ autoantibody was tested in a panel alongside autoantibodies to PGP 9.5 and annexin I, and together these displayed a sensitivity of 55% and specificity of 95%. Furthermore, reactivity to laminin receptor 1 (LAMR1) has also shown high reactivity to lung cancer patient sera (88). This protein microarray study by Qiu et al. tested 85 pre-diagnostic lung cancer patients and 85 matched healthy controls against 14-3-3 Θ, LAMR1 and annexin I and achieved a sensitivity of 51% and a specificity of 82% (88).

Yang et al. (89) analysed a study cohort consisting of 40 newly diagnosed lung squamous carcinoma patients, 30 patients with various other types of cancer and 50 healthy controls and performed two-dimensional electrophoresis (2D-PAGE) and an enzyme-linked immunosorbent assay (ELISA) to identify triose-phosphate isomerase (TPI) and mitochondrial superoxide dismutase 2 (MnSOD) autoantibodies as potential early-stage lung cancer diagnostic biomarkers with a sensitivity of 47% and a specificity of 90%.

Furthermore, research by He et al. (90), utilised a combination of methods including 2D-PAGE, western blotting, mass spectrometry and ELISA to identify further reactivity and therefore autoantibody production to alpha enolase1 (α-enolase) in 28% of lung cancer patients. When α-enolase was used in combination with other potential autoantibody biomarkers such as cancerembryonic antigen (CEA) and cytokeratin fragment 21-1 (CYFRA 21-1) in a cohort of 94 non-small cell lung cancer patients, 15 small-cell lung cancer patients,
10 gastric cancer patients, 8 colon cancer patients, 9 Mycobacterium avium complex infection of the lung patients and 60 healthy controls, the sensitivity of this potential diagnostic lung cancer biomarker panel was calculated to be as high as 69.3% with a specificity 98.3% (90). An ELISA panel of potential diagnostic lung cancer autoantibody biomarkers comprised of p53, c-myc, Her-2, NY-ESO-1, MUC1, cancer antigen 1 (CAGE) and Tumour-associated antigen GBU4-5 (GBU4-5) tested by Chapman et al., yielded promising results of 76% sensitivity and 92% specificity in another cohort consisting of 82 non-small cell lung cancer patients, 22 small-cell lung cancer patients and 50 healthy controls (91).

### 4.4) Colon cancer

To date, CEA is the only serological biomarker in clinical use for the diagnosis of colorectal cancer; however, this biomarker is also hindered by its low specificity and sensitivity (92). A study by Liu et al. (92) showed an increase in colon cancer detection sensitivity over CEA when an ELISA based mini-array containing five tumour-associated antigens, IMP dehydrogenase 1 (Imp1), nucleoporin p62 (p62), K homology domain containing protein over expressed in cancer (Koc), p53 and c-myc, were used. When 46 colon cancer patients and 58 healthy controls were probed with the above mentioned mini-array, the sensitivity for the combined panel was 82.6% and its specificity was 89.7% in the colon cancer patients (92). Autoantibodies to the FAS receptor (Fas/CD95) (93) also show specificity for the early detection of colon cancer. Reipert et al. (93) investigated sera from 38 healthy controls, 38 patients with colorectal adenomas and 21 patients with colorectal adenocarcinoma in their ELISA-based array for reactivity against Fas and did not detect any reactivity with Fas in the sera of healthy controls. Furthermore, the anti-Fas antibody titers were higher in patients with colorectal adenomas compared to colorectal adenocarcinoma patient anti-Fas titers resulting in sensitivity and specificity of this array of 17% and 100% for colon cancer respectively (93), making this biomarker a good option to confirm negative disease status but not to confirm
positive disease status and thus the search for colon cancer biomarkers is still ongoing.

Another marker called Mucin-5AC (MUC5AC), was investigated in order to increase sensitivity of colon cancer detection. This ELISA-based experiment was performed on 20 patients with colorectal polyps, 30 colorectal cancer patients and 22 healthy volunteers and it’s sensitivity was found to be 54%, however this marker exhibited a much lower specificity than Fas of 73% (94).

Studies have shown that autoantibodies to p53 can help identify individuals at increased risk of developing colorectal cancer since these autoantibodies have been detected in patients with precancerous colorectal cancer lesions. In fact, the screening for these autoantibodies is suggested in addition to colonoscopy screens (95-97). However, antibodies to p53 have also been associated with a range of other cancers, which reduces the specificity of this biomarker for colon cancer.

Another study by He et al. (98) has shown increased levels of autoantibodies to HSP60 in the sera of 13 out of 25 colorectal cancer patients relative to 1 out of 15 healthy volunteer sera which results in 52% sensitivity and 93.3% specificity of this marker for colon cancer diagnosis; however, the same autoantibodies have also been observed in breast cancer patients which demonstrates that this biomarker is not specific to colon cancer alone (98). Research by Chen et al. (99) investigating the reactivity to nucleobindin 1 (Calnuc) in sera from 52 colon cancer patients, 39 breast cancer patients, 16 cervical cancer patients, 70 esophageal cancer patients, 73 gastric cancer patients, 62 hepatic cancer patients, 104 lung cancer patients, 14 nasopharyngeal cancer patients, 17 ovarian cancer patients and 82 healthy controls, showed no significantly higher Calnuc frequency in various cancer groups (4.7%) to healthy individuals (1.2%). When colon cancer patients were investigated Calnuc frequency was detected to be 11.5% in patients which is significantly higher than the frequency mentioned in controls. The same study achieved an increase to 65.4% sensitivity and 93.9% specificity
when Calnuc was added to a tumour-associated antigen panel comprised of c-myc, p53, G2/mitotic-specific cyclin-B1 (CCNB1) and G1/S-specific cyclin-D1 (CCND1) (99).

### 4.5) Stomach cancer

To date, there are no stomach cancer specific biomarkers although p53 autoantibodies have been identified as being associated with stomach cancer as well as several other cancers (100, 101). Previously, Shimizu et al., (101) tested the sera of 40 gastric cancer patients after gastric resection for the presence of p53, CEA and CA 19-9 autoantibodies. This ELISA-based assay showed that 15% of the patients were positive for p53 autoantibody but not for CEA or Ca 19-9 and 17.5% were positive for CEA only while 10% were positive for CA 19-9 (101). Patients seemed to express either p53 autoantibodies or CEA and Ca 19-9 autoantibodies. When all three markers were applied as a panel, a panel sensitivity of 42.5% was achieved which was deemed too low for the panel to be utilised in the diagnosis of gastric cancer (101). Three years later, Qiu et al., (100) tested 61 pre-operative patients with gastric carcinoma and 30 patients with other gastric diseases including 10 patients with gastritis, 10 patients with gastric ulcers and 10 patients with gastro spasm against a combined panel of CEA and p53 autoantibodies. This panel showed positive reactivity for these two markers in 31 out of the 61 gastric carcinoma patient sera, indicating a sensitivity of 50.8%, but did not show positive reactivity with sera from any of the other gastric diseases (100). Although this panel yielded higher sensitivity, it is important to keep in mind that this panel was tested against pre-operative gastric cancer patients while Shimizu et al. (101) tested post-gastric resection patients, suggesting once more that the autoantibody profile could have been attenuated on removal of the "immunogen" after treatment. The GastroPanel™, utilised to detect gastric mucosa variations including atrophic gastritis, incorporates the biomarkers serum pepsinogen I (PGA1) and serum pepsinogen II (PGA2), gastrin-17 as well as antibodies against *Helicobacter pylori*. Since most stomach cancers arise from chronic
inflammations such as gastritis (102), GastroPanel™ may aid in the early-stage diagnosis of the cancer or may also aid in the identification of individuals who may be at increased risk of developing stomach cancer once inflammation of their gastric mucosal wall has been confirmed.

4.6) Liver cancer

Hepatocellular carcinoma (HCC), the predominant form of primary liver cancer, is diagnosed by the histological examination of the liver using ultrasonography (103). Although this technology displays a sensitivity of 60-80%, a positive predictive value of 78% and a specificity of up to 98% (104), it is nonetheless subject to detection bias as it is an operator-dependent technology and small tumours may be overlooked against a cirrhotic background (105, 106). Therefore, there is a need to support the diagnosis of this cancer on a more molecular level. The search for autoantibodies for the diagnosis of the cancer is therefore of great interest in order to develop a blood test for HCC diagnosis.

Alpha-fetoprotein (AFP), a normal serum protein synthesised during embryonic development, is currently considered to be the best biomarker available for HCC diagnosis (107). Elevated levels of AFP are observed in pregnant woman and chronic liver disease patients; however, lower levels of this biomarker are also observed in healthy individuals and non-pregnant woman, implying that AFP cannot be utilised for the diagnosis of small HCC tumours (108). The sensitivity of the biomarker lies between 40-65% and its specificity between 75-90% while displaying a positive predictive value of only 12% (109). One major study by Zhang et al. (110) was performed in China to measure whether a combination of routine ultrasonography screening and an ELISA-based AFP test (cut-off value at 20 µg/l) increases HCC detection rates. Out of the 18816 people with hepatitis B virus infection included in this study, 9373 were randomly selected to be part of the screening group, which was offered an
ultrasonography examination and an AFP test combination every 6 months for a period of up to 5 years and the remaining 9443 people were randomly selected to be part of the control group which did not receive any extra screening but continued to use health-care facilities (110). During this study, 71 cases of HCC were detected in the screening group compared to 67 in the control group (110) but this slight increase was not considered to be sufficient evidence to support further use of AFP testing in combination with routine ultrasonography examination and therefore routine ultrasonography examination alone is used during clinical practice(107). In 2006, Farinati et al. (109) tested 1158 patients with HCC for AFP levels in their ELISA-based test. AFP levels <20ng/ml were considered normal, while 21-400ng/ml was defined as elevated and >400 ng/ml were considered as diagnostically significant. With regards to these levels, the group confirmed the low sensitivity of AFP as 54% and did not recommend this marker for utilisation in the routine diagnosis of HCC (109). Serum levels of des-gamma-carboxyprothrombin (DCP), another potential biomarker for HCC diagnosis have been compared to AFP levels in an ELISA-based experiment performed by Marrero et al. (111). This research tested sera from 48 healthy controls, 51 patients with noncirrhotic hepatitis, 53 patients with compensated cirrhosis and 55 patients with hepatocellular carcinoma against DCP and AFP individually and in combination in order to find the best marker or panel to differentiate hepatocellular carcinoma patients from other non-malignant chronic liver disease patients. The study concluded that the sensitivity and specificity of AFP levels alone are 77% and 73%, and of DCP 89% and 95% respectively and the combination of the two markers resulted in 88% and 95% sensitivity and specificity (111).

The utilisation of SEREX methodology showed the presence of Hepatocellular carcinoma-associated antigen HCC-22-5 (HCC-22-5) autoantibodies in 78.9% patients with liver cancer who were diagnosed as AFP-negative and these autoantibodies were not detected in healthy control sera nor in the sera of lung or gastrointestinal cancer patients (112).
SEREX-based study, Takashima et al. (113) tested 15 hepatocellular carcinoma patients and 20 healthy control sera against Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), heat shock 70kDa protein 1 (HSP70), MnSOD and peroxiredoxin (Prx) and found that high GAPDH autoantibody levels were present in 33.3% of patients and in 35% of controls, indicating that routine use of GAPDH for HCC diagnosis is not recommended while high HSP70 levels were detected in 46.7% of patients and in only 10% of controls.(113). In the same study, high serological autoantibody levels of MnSOD were detected in 40% of patient sera and in only 10% of controls while high PRX autoantibody levels were detected in 33.3% of patients and 0% of controls (113).

Chronic hepatitis B virus (HBV) infection and cirrhosis are high risk factors for the development of HCC and tumour associated antigen autoantibodies can be found in HBV-associated HCC patients(107, 114). SERPA and protein microarray studies have found autoantibodies to proteins including: EEF2, heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2), DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked (DDX3X), apoptosis inducing factor (AIF), prostatic binding protein (PBP) and TIP to be significantly higher in HCC patients than in healthy individuals or chronic hepatitis patients. The sensitivity of any of the four markers: DDX3X, PBP, EEF2 and AIF was found to be 50-85% and increased to 90% when analysed as a biomarker panel (115).

5) Potential future use of autoantibodies as diagnostic cancer biomarkers

By avoiding the progression of a cancer to an often incurable metastatic stage, early detection of all cancers may lead to increased survival rates and better quality of life. The golden standard diagnostic techniques utilised today, such as mammography for breast cancer detection, are highly successful, however, they are often subject to detection bias and may
result in false negative diagnosis of a patient whose tumour has been overlooked due to the limitations of current diagnostic techniques. To aid the early detection of all cancers and to ensure that all oncology patients are correctly diagnosed, the focus now lies in finding biomarkers, indicating a positive diagnosis at an earlier stage. This early detection of any cancer will potentially aid health professionals to choose an appropriate therapeutic intervention which will target early-stage tumours at their most treatable stage.

Levels of certain autoantibodies have been found to arise prior and during tumour formation, indicating that autoantibodies may serve as highly effective biomarkers for the early diagnosis of cancers. In order to search for such autoantibodies, several state-of-the art technologies and methodologies have been developed, including the serological analysis of tumour antigens by recombinant cDNA expression cloning (SEREX), phage display, protein microarrays, reverse-capture microarrays, serological proteome analysis (SERPA) and multiple affinity protein profiling (MAPPing). These methodologies and techniques have enabled the simultaneous identification of several autoantibodies for different cancers and these are currently being tested for their potential to serve as diagnostic biomarkers for specific cancers. So far, the clinical application of most identified autoantibodies has been hindered by their low sensitivity, specificity and predictive value percentages as well as poor reproducibility within different experimental designs and applications of the methodology.

Nonetheless, the number of autoantibodies identified that displayed improved sensitivity, specificity and predictive value percentages has been increasing and several studies have shown increases in sensitivity and specificity scores when the potential autoantibodies are applied in combination as in a diagnostic biomarker panel. As previously stated in this review, PSA was the only marker used for prostate cancer diagnosis and its use has now been discontinued due to low sensitivity scores. Research by O’Rouke et al. (74) tested a study cohort of 80 samples for reactivity against PSA alone in comparison with a new biomarker
panel including markers TLN1, TARDBP, LEDGF, CALD1 and PARK7. The research showed an increase in sensitivity from 12.2% for PSA alone to 95% for the panel while specificity was calculated to be 80% in both PSA alone and the panel. This research is an example of the discovery of combined panels of markers that show potential as biomarker panels for the diagnosis of prostate cancer. On the other hand, Yi et al., (80) discovered a single potential diagnostic biomarker called alpha-2-HS-glycoprotein for breast cancer diagnosis. This marker yielded the high sensitivity of 79% for breast cancer detection.

Chapman et al. (91) also showed that a multi-marker panel, analysed via enzyme-linked immunosorbent assay, was informative for the early diagnosis of lung cancer. This panel included the markers p53, c-myc, HER2, MUC1, NY-ESO-1, CAGE and GBU4-5 and resulted in 76% sensitivity and 92% specificity, scores that are far above those achieved by previous lung cancer-associated diagnostic autoantibody biomarker studies. Another panel discovered by Liu et al. (92) for the diagnosis of colon or colorectal cancer achieved 82.6% and 89.7% sensitivity and specificity. The panel consists of the markers CEA, Imp-1, p62, Koc, p53 and c-myc. Furthermore, Qiu et al. (100) demonstrated an increase in sensitivity and specificity to 50.8% and 100% respectively when p53 and CEA were tested in combination for the diagnosis of stomach or gastric cancer. Finally, Marrero et al. (111) also performed an enzyme-linked immunosorbent assay and demonstrated that the single marker, DCP, has the highest diagnostic potential for the early detection of liver cancer due to its high sensitivity of 89% and specificity of 95%.

In the future, more diagnostic cancer biomarker studies are required that contain larger cohorts to avoid intersample variations. Furthermore consistent methodological conditions for autoantibody detection are essential. Further autoantibody biomarker research may provide new knowledge of molecular events in carcinogenesis and cancer progression thus improving early detection of individuals at risk of disease recurrence.
Acknowledgements

The authors thank Johan Poole-Johnson and staff at Oxford Gene Technology, Oxford, UK for their assistance with this review.
References


<table>
<thead>
<tr>
<th>methodology name</th>
<th>high-throughput</th>
<th>cost</th>
<th>time</th>
<th>advantages</th>
<th>disadvantages</th>
</tr>
</thead>
</table>
| SEREX            | Yes             |      |      | • may take several days  
• This is the most time consuming of all the methods due to the need to construct the cDNA library  
• allows detection from in-vivo material  
• use of multi-antigen specific patient serum allows the identification of several tumour-specific antigens in one experiment  
• both the tumour-specific antigen and it's coding cDNA are present in the same plaque when immunoscreening is performed which allows the subsequent sequencing of the matching cDNA immediately slightly more sensitive than SERPA  | • high likelihood of false-positive results  
• does not detect alternate tumour-associated post-translational modifications of antigens  
• use of tumour tissue from a single cancer followed by screening with autologous patient serum limits identification of tumour-associated antigens to that of a single patient  
• parallel analysis of tumour proteins with healthy donor sera as controls cannot be performed easily |
<p>| phage display    | Yes - higher throughput than SEREX | more cost-effective if phage proteins are fused to antigens on bacteriophage surface | may take several days | constructed directly from tumour tissue or patient tumour material derived cell line | does not detect alternate tumour-associated post-translational modifications of antigens |</p>
<table>
<thead>
<tr>
<th>Protein Microarray</th>
<th>Yes</th>
<th>Production of thousands of recombinant proteins is very expensive</th>
<th>Time restriction due to short shelf-life of protein arrays</th>
<th>Large numbers of antigens can be tested against large numbers of sera in a single test</th>
<th>High quality protein synthesis is required</th>
<th>Other than high quality antibodies or antigens, only commercially available proteins can be studied</th>
<th>Time restriction due to short shelf-life of protein arrays</th>
<th>High reproducibility is difficult to achieve</th>
<th>Enormous data collection requires specialised software tools</th>
<th>Production of thousands of recombinant proteins is very labour-intensive</th>
<th>Recombinant proteins produced in non-mammalian systems may not have the correct post-translational modifications and may therefore be misfolded</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERPA</td>
<td>Yes</td>
<td>More cost-effective than SEREX</td>
<td>May be completed within hours</td>
<td>Effective separation of a complex mixture of proteins based on their isoelectric points and molecular weights</td>
<td>Limited identification of low-abundance and transmembrane tumour-associated antigens</td>
<td>Due to the use of western blots only linear epitopes can be detected</td>
<td>Separation of cell membrane proteins remains a challenge due to their insoluble nature in aqueous buffers</td>
<td>This method of autoantibody detection is very labour-intensive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liquid-based separations are amenable to automation and the ELISA format can be coupled to mass spectrometric analysis which increases the throughput</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table:**

- **Protein Microarray:**
  - Yes
  - Production of thousands of recombinant proteins is very expensive
  - Time restriction due to short shelf-life of protein arrays
  - Large numbers of antigens can be tested against large numbers of sera in a single test
  - Purified, recombinant or synthetic proteins may be used
  - Array platform may be two- or three-dimensional
  - Yeast or insect cells may be used as alternative expression systems to produce libraries with correct post-translational modifications
  - 3-D structure is often intact optimising antigen-antibody interaction for recombinant proteins produced in mammalian systems
  - Requires only minute amounts of sera

- **SERPA**
  - Yes
  - Liquid-based separations are amenable to automation and the ELISA format can be coupled to mass spectrometric analysis which increases the throughput
  - May be completed within hours
  - Effective separation of a complex mixture of proteins based on their isoelectric points and molecular weights
  - Allows detection from in-vivo material
  - Allows for the identification of tumour-specific post-translational modifications and isoforms
  - Avoids the time-consuming construction of cDNA libraries
  - Parallel analysis of tumours
  - Limited identification of low-abundance and transmembrane tumour-associated antigens
  - Due to the use of western blots only linear epitopes can be detected
  - Separation of cell membrane proteins remains a challenge due to their insoluble nature in aqueous buffers
  - This method of autoantibody detection is very labour-intensive
| MAPPing | Yes | similar cost-efficiency to SERPA | • may be completed within hours | • tumour antigens are maintained in solution which allows the identification of structural epitopes | • restricted tumour antigen identification to antibody interactions with a low dissociation rate constant | • limited detection of tumour antigens in more complex protein solutions due to the use of immunoprecipitation |

proteins with healthy donor sera as controls can be performed easily

- 2-D immunoblots provide a global view of the antibody–tumour-associated antigen interaction
Table 2: List of studies mentioned in this review including the study sample size, methods utilised, biomarker abbreviation, full biomarker names, cancer sites associated with biomarker for possible diagnosis, encoding genes, alternative names and the accuracy of individual biomarkers or biomarker panels are provided. Only cancer types described in this review are included.

<table>
<thead>
<tr>
<th>REFERENCE</th>
<th>SAMPLE SIZE</th>
<th>METHODS UTILISED</th>
<th>PROTEIN ABBREVIATION</th>
<th>PROTEIN FULL NAME</th>
<th>CANCER ASSOCIATED WITH PROTEIN FOR POSSIBLE DIAGNOSIS</th>
<th>ENCODING GENE</th>
<th>ALTERNATIVE NAMES</th>
<th>ACCURACY OF MARKER/MARKER PANEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xie et al., 2011 [73]</td>
<td>131 pre surgery biopsy confirmed prostate cancer patients and 121 prostatitis and/or benign prostatic hyperplasia patients total cohort = 252</td>
<td>novel seroMAP-based multiplex &quot;A+PSA&quot; (autoantibody + PSA) assay versus PSA assay alone</td>
<td>NY-ESO-1</td>
<td>New York oesophageal squamous cell carcinoma</td>
<td>Prostate, Breast, Lung</td>
<td>NY-ESO-1</td>
<td></td>
<td>sensitivity = 79% specificity = 84%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SSX-2,4</td>
<td>synovial sarcoma, X breakpoint 2,4</td>
<td>Prostate</td>
<td>SSX2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>XAGE-1b</td>
<td>X antigen family, member 1B</td>
<td>Prostate</td>
<td>XAGE1B</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LEDGF</td>
<td>lens epithelium-derived growth factor p75</td>
<td>Prostate</td>
<td>PSIP1</td>
<td></td>
<td>PSIP1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AMACR</td>
<td>alpha-methylacyl-CoA racemase</td>
<td>Prostate</td>
<td>AMACR</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p90</td>
<td>transferrin receptor protein 90</td>
<td>Prostate</td>
<td>TFRC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PSA</td>
<td>prostate-specific antigen</td>
<td>Prostate</td>
<td>CLK3</td>
<td>KLK3, gamma-seminoprotein</td>
<td>sensitivity = 52% specificity = 79%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PSA</td>
<td>prostate-specific antigen</td>
<td>Prostate</td>
<td>CLK3</td>
<td>KLK3, gamma-seminoprotein</td>
<td>sensitivity = 81.6% specificity = 88.2%</td>
</tr>
<tr>
<td>Wang et al., 2005 [14]</td>
<td>119 prostate cancer patients and 138 healthy controls total cohort = 257</td>
<td>phage protein microarray</td>
<td>BRD2</td>
<td>bromodomain-containing protein 2</td>
<td>Prostate</td>
<td>BRD2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>eIF4G1</td>
<td>eukaryotic translation initiation factor 4 gamma 1</td>
<td>Prostate</td>
<td>eIF4G1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RPL22</td>
<td>ribosomal protein L22</td>
<td>Prostate</td>
<td>RPL22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RPL13a</td>
<td>ribosomal protein L8a</td>
<td>Prostate</td>
<td>RPL13A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pubs</td>
<td>patients</td>
<td>method</td>
<td>protein</td>
<td>source</td>
<td>cohort size</td>
<td>sensitivity</td>
<td>specificity</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>----------</td>
<td>--------</td>
<td>---------</td>
<td>--------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>1. O’Rouke et al., 2012 [74]</td>
<td>41 prostate cancer patients and 39 benign prostate hyperplasia patients total cohort = 80</td>
<td>reverse capture microarray</td>
<td>XP_373908 hypothetical protein XP_373908</td>
<td>Prostate</td>
<td>Prostate</td>
<td>KLK3</td>
<td>KLK3, gamma-semioneprotein</td>
<td>sensitivity = 12.2% specificity = 80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PSA prostate-specific antigen</td>
<td>Prostate</td>
<td></td>
<td>TLR1</td>
<td>TLR1</td>
<td>sensitivity = 12.2% specificity = 80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TARDBP TAR DNA-binding protein</td>
<td>Prostate</td>
<td></td>
<td>TARDBP</td>
<td>TARDBP</td>
<td>sensitivity = 12.2% specificity = 80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LEDGF lens epithelium-derived growth factor p75</td>
<td>Prostate</td>
<td></td>
<td>PSIP1</td>
<td>PSIP1</td>
<td>sensitivity = 95% specificity = 80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CALD1 Caldesmon</td>
<td>Prostate</td>
<td></td>
<td>CALD1</td>
<td>CALD1</td>
<td>sensitivity = 95% specificity = 80%</td>
</tr>
<tr>
<td>2. Looi et al., 2006 [81]</td>
<td>479 patients with various types of cancer (including 41 breast cancer sera) and 82 healthy controls total cohort = 561</td>
<td>ELISA, western blotting</td>
<td>c-myc avian myelocytomatosis viral oncogene homolog</td>
<td>Breast, Lung, Colon</td>
<td>Myc</td>
<td>Myc</td>
<td>sensitivity = 43.9% specificity = 97.6%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p16 protein 16</td>
<td>Breast, Colon</td>
<td>p16</td>
<td>p16</td>
<td>sensitivity = 43.9% specificity = 97.6%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p53 (tumour) protein 53</td>
<td>Breast, Lung, Colon, Stomach, Liver</td>
<td>TP53</td>
<td>TP53</td>
<td>sensitivity = 43.9% specificity = 97.6%</td>
<td></td>
</tr>
<tr>
<td>3. Zhong et al., 2008 [82]</td>
<td>87 breast cancer patients and 87 healthy controls total cohort = 174</td>
<td>ELISA, SEREX</td>
<td>SERAC1 serine active site containing 1</td>
<td>Breast</td>
<td>SERAC1</td>
<td>SERAC1</td>
<td>sensitivity = 77% specificity = 82.8%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RELT receptor expressed in lymphoid tissues</td>
<td>Breast</td>
<td>RELT</td>
<td>RELT</td>
<td>sensitivity = 77% specificity = 82.8%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ASB-9 ankyrin repeat and suppressor of cytokine signalling (SOCS) box protein 9</td>
<td>Breast</td>
<td>ASB9</td>
<td>ASB9</td>
<td>sensitivity = 77% specificity = 82.8%</td>
<td></td>
</tr>
<tr>
<td>Author</td>
<td>Study Details</td>
<td>Assay</td>
<td>Marker</td>
<td>Tissues</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>---------------</td>
<td>-------</td>
<td>--------</td>
<td>---------</td>
<td>-------------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desmetz et al., 2008 [83]</td>
<td>49 ductal carcinoma in situ patients, 58 early-stage breast cancer patients, 20 patients with other types of cancer, 20 patients with various autoimmune diseases and 93 healthy controls total cohort = 240</td>
<td>ELISA, SERPA</td>
<td>HSP60 heat shock protein 60</td>
<td>Breast, Colon</td>
<td>HSP60</td>
<td>sensitivity = 31.8% specificity = 95.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chapman et al., 2007 [84]</td>
<td>40 ductal carcinoma in situ patients, 97 primary breast cancer patients and 94 healthy controls total cohort = 231</td>
<td>ELISA</td>
<td>p53 (tumour) protein 53</td>
<td>Breast, Lung, Colon, Stomach, Liver</td>
<td>TP53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c-myc avian myelocytomatosis viral oncogene homolog</td>
<td>Breast, Lung, Colon</td>
<td>Myc</td>
<td>Myc</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HER2 human epidermal growth factor receptor 2</td>
<td>Breast, Lung</td>
<td>ERBB2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NY-ESO-1 New York oesophageal squamous cell carcinoma</td>
<td>Prostate, Breast, Lung</td>
<td>NY-ESO-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BRCA1 breast cancer type 1 susceptibility protein</td>
<td>Breast</td>
<td>BRCA1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BRCA2 breast cancer type 2 susceptibility protein</td>
<td>Breast</td>
<td>BRCA2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MUC1 Mucin 1, cell surface associated</td>
<td>Breast, Lung</td>
<td>MUC1</td>
<td>PEM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Patient/controls</td>
<td>Methodologies</td>
<td>Protein/Proteins</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------------------------------------------------------</td>
<td>------------------------------------------</td>
<td>------------------------------------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamrita et al., 2008 [85]</td>
<td>40 patients with invasive breast cancer and 42 healthy controls</td>
<td>SERPA, HSP60, heat shock protein 60, Breast, Colon</td>
<td>HSP60, breast, colon</td>
<td>47.5%</td>
<td>95.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yi et al., 2009 [80]</td>
<td>81 pre-surgery breast cancer patients and 73 healthy controls</td>
<td>2-DE, immunoblot, mass spectrometry</td>
<td>AHSG, alpha-2-HS-glycoprotein, AHSG, fetuin-A</td>
<td>79%</td>
<td>90.4%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pereira-Faca et al., 2007</td>
<td>45 newly diagnosed lung cancer patients, 18 prediagnostic lung cancer patients and 62 matched healthy controls</td>
<td>1D-SDS-PAGE, 2D-PAGE, Western blotting, mass spectrometry</td>
<td>14-3-3 θ, 14-3-3 theta, YWHAQ, ANXA1, lipocortin I</td>
<td>55%</td>
<td>95%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qiu et al., 2008 [88]</td>
<td>85 prediagnostic lung cancer patients and 85 matched healthy controls</td>
<td>protein microarray</td>
<td>ANXA1, annexin I, ANXA1, lipocortin I</td>
<td>51%</td>
<td>82%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Total Cohort</td>
<td>Other Conditions</td>
<td>Biomarkers</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yang et al., 2007 [89]</td>
<td>120</td>
<td>40 newly diagnosed lung squamous carcinoma patients, 30 patients with various other types of cancer and 50 healthy controls</td>
<td>2D-PAGE, ELISA, triose-phosphate isomerase (Lung)</td>
<td>47.5%</td>
<td>90%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>He et al., 2007 [90]</td>
<td>196</td>
<td>94 non-small cell lung cancer patients, 15 small-cell lung cancer patients, 10 gastric cancer patients, 8 colon cancer patients, 9 Mycobacterium avium complex infection of the lung patients and 60 healthy controls</td>
<td>2D-PAGE, Western blotting, mass spectrometry, ELISA, carcinoembryonic antigen (Breast, Colon, Lung, Stomach), CEACAM genes</td>
<td>69.3%</td>
<td>98.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chapman et al., 2008 [91]</td>
<td>154</td>
<td>82 non-small cell lung cancer patients, 22 small-cell lung cancer patients and 50 healthy controls</td>
<td>ELISA, p53 (tumour) protein 53, avian myelocytomatosis viral oncogene homolog, human epidermal growth factor</td>
<td>76%</td>
<td>92%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liu et al., 2009 [92]</td>
<td>receptor 2</td>
<td>46 colon cancer patients and 58 healthy controls</td>
<td>total cohort = 104</td>
<td>ELISA mini-array</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------</td>
<td>---------------------------------</td>
<td>------------------</td>
<td>-----------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUC1</td>
<td>Mucin 1, cell surface associated.</td>
<td>Breast, Lung.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAGE</td>
<td>Cancer antigen. 1</td>
<td>Lung.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBU4-5</td>
<td>Tumour-associated antigen GBU4-5</td>
<td>Lung.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imp 1</td>
<td>IMP dehydrogenase 1</td>
<td>Colon.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p62</td>
<td>Nucleoporin p62</td>
<td>Colon, Liver.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p53</td>
<td>(tumour) protein 53</td>
<td>Breast, Lung, Colon, Stomach, Liver.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity = 82.6% Specificity = 89.7%
<table>
<thead>
<tr>
<th>Study</th>
<th>Number of Patients</th>
<th>Tests / Techniques</th>
<th>Tumors / Organs</th>
<th>Biomarkers / Receptors</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reipert et al., 2005</td>
<td>97</td>
<td>ELISA</td>
<td>Colon</td>
<td>Fas/CD95, FAS receptor</td>
<td>17%</td>
<td>100%</td>
</tr>
<tr>
<td>Kocer et al., 2006</td>
<td>72</td>
<td>ELISA</td>
<td>Colon</td>
<td>MUC5AC, Mucin-SAC</td>
<td>54%</td>
<td>73%</td>
</tr>
<tr>
<td>He et al., 2007</td>
<td>40</td>
<td>SERPA, 2D-PAGE, ELISA, western blotting, mass spectrometry, immunohistochemistry</td>
<td>Breast, Colon</td>
<td>HSP60, heat shock protein 60</td>
<td>52%</td>
<td>93.3%</td>
</tr>
<tr>
<td>Chen et al., 2007</td>
<td>104</td>
<td>ELISA, western blotting, immunohistochemistry</td>
<td>Breast, Colon, Stomach, Lung</td>
<td>c-myc, avian myelocytomatosis viral oncogene homolog, Myc, p53</td>
<td>65.4%</td>
<td>93.9%</td>
</tr>
<tr>
<td>Study</td>
<td>Patient Group</td>
<td>Biomarkers</td>
<td>Tissues</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
<td>--------------------------</td>
<td>---------------</td>
<td>---------------</td>
<td></td>
</tr>
<tr>
<td>Shimizu et al., 2005</td>
<td>40 gastric cancer patients who had undergone gastric resection</td>
<td>TP53 53 (tumour) protein 53</td>
<td>Breast, Lung, Colon, Stomach, Liver</td>
<td>42.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>total cohort = 40</td>
<td>CEA carinoembryonic antigen</td>
<td>Breast, Colon, Lung, Stomach</td>
<td></td>
<td>MECAAM genes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 19-9 carbohydrate antigen 19-9</td>
<td>Stomach</td>
<td></td>
<td>MUC1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cancer antigen 19-9, sialylated Lewis (a) antigen</td>
<td></td>
</tr>
<tr>
<td>Qiu et al., 2007</td>
<td>61 pre-operative gastric carcinoma patients, 10 patients with gastritis, 10</td>
<td>TP53 53 (tumour) protein 53</td>
<td>Breast, Lung, Colon, Stomach, Liver</td>
<td>50.8%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>patients with gastric ulcers and 10 patients with gastrospasm</td>
<td>CEA carinoembryonic antigen</td>
<td>Breast, Colon, Lung, Stomach</td>
<td></td>
<td>MECAAM genes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>total cohort = 91</td>
<td></td>
<td></td>
<td></td>
<td>MUC1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cancer antigen 19-9, sialylated Lewis (a) antigen</td>
<td></td>
</tr>
<tr>
<td>Farinati et al., 2006</td>
<td>1158 patients with Hepatocellular carcinoma</td>
<td>AFP alpha-fetoprotein</td>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>total cohort = 1158</td>
<td></td>
<td></td>
<td></td>
<td>α-fetoprotein, alpha-1-fetoprotein, alpha-fetoglobulin</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Cohort Description</td>
<td>ELISA Assays</td>
<td>Liver Functions</td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>--------------</td>
<td>----------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td>Marrero et al., 2003</td>
<td>15 healthy controls and 20 patients with hepatocellular carcinoma; total cohort = 35</td>
<td>ELISA</td>
<td>Liver</td>
<td>77%</td>
<td>73%</td>
<td></td>
</tr>
<tr>
<td>Takashima et al., 2006</td>
<td>15 hepatocellular carcinoma patients and 20 healthy controls; total cohort = 35</td>
<td>2-DE, 2-D immunoblot, SEREX</td>
<td>Liver</td>
<td>46.7%</td>
<td>90%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-DE, 2-D immunoblot, SEREX</td>
<td>ELISA</td>
<td>Liver</td>
<td>88%</td>
<td>95%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-DE, 2-D immunoblot, SEREX</td>
<td>ELISA</td>
<td>Liver</td>
<td>89%</td>
<td>95%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-DE, 2-D immunoblot, SEREX</td>
<td>ELISA</td>
<td>Liver</td>
<td>40%</td>
<td>90%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-DE, 2-D immunoblot, SEREX</td>
<td>ELISA</td>
<td>Liver</td>
<td>33.3%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-DE, 2-D immunoblot, SEREX</td>
<td>ELISA</td>
<td>Liver</td>
<td>33.3%</td>
<td>65%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-DE, 2-D immunoblot, SEREX</td>
<td>ELISA</td>
<td>Liver</td>
<td>77%</td>
<td>73%</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Count of Cohort</td>
<td>Methodology</td>
<td>Antigen/Gene</td>
<td>Tissue(s)</td>
<td>Specificity</td>
<td>Sensitivity</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------------------------------------------</td>
<td>---------------------------------------</td>
<td>---------------------------------------------------</td>
<td>-----------</td>
<td>-------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Zhou et al., 2005</td>
<td>128 hepatocellular carcinoma patients, 76 chronic hepatitis patients, 22 liver cirrhosis patients, 54 nasopharynx cancer patients, 54 gastric-intestine patients and 80 healthy controls total cohort = 421</td>
<td>SEREX, western-blot, ELISA</td>
<td>HCC-22-5 Hepatocellular carcinoma-associated antigen HCC-22-5</td>
<td>Liver</td>
<td>HCC</td>
<td>specificity = 100% sensitivity = 40%</td>
</tr>
<tr>
<td>Li et al., 2008</td>
<td>174 HCC patients, 63 chronic hepatitis patients, 66 patients with other types of cancer and 71 healthy controls total cohort = 374</td>
<td>SERPA, 2-DE, western blotting, protein microarray</td>
<td>DDX3X DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked</td>
<td>Liver</td>
<td>DDX3X</td>
<td>specificity = 69.8% sensitivity = 85.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AIF apoptosis inducing factor</td>
<td>Liver</td>
<td>AIF</td>
<td>sensitivity = 81.4% specificity = 55.9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EEF2K eukaryotic elongation factor 2 kinase</td>
<td>Breast, Liver</td>
<td>EEF2K</td>
<td>sensitivity = 78.5% specificity = 78.8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PBP prostatic binding protein</td>
<td>Liver</td>
<td>PBP</td>
<td>sensitivity = 82.6% specificity = 48.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>hnRNP A2 heterogeneous nuclear ribonucleoprotein A2</td>
<td>Liver</td>
<td>HNRNPA2</td>
<td>sensitivity = 70.9% specificity = 64.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TPI triose-phosphate isomerase</td>
<td>Lung, Liver</td>
<td>TPI</td>
<td>sensitivity = 75% specificity = 64.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AFP alpha-fetoprotein</td>
<td>Liver</td>
<td>AFP</td>
<td>sensitivity = 75% specificity = 72.9%</td>
</tr>
</tbody>
</table>

**Note:** The table reflects the detection of biomarkers for different cancer types and their associated sensitivities and specificities. The data is derived from the referenced studies and methods.
Figure 1

Technologies utilised for autoantibody discovery

- **SEREX** serological analysis of tumour antigens by recombinant cDNA-expression cloning
- **Phage display**
  - cDNA-phage display library
  - tumour/cell lysate
  - purified or recombinant proteins
  - antibody array
  - arrayed on slides
- **Protein array**
  - tumour/cell lysate
  - target cDNA
  - two-dimensional liquid chromatography
  - immunoblot
- **SERPA** serological proteome analysis
  - tumour/cell lysate
  - two-dimensional electrophoresis
  - immunoblot
- **MAPPING** multiple affinity protein profiling
  - tumour/cell lysate
  - two-dimensional immunoefficiency

Probe with patient and control sera

Autoantibody identification using tandem mass spectrometry
Serological Autoantibodies as Diagnostic Cancer Biomarkers - a review

Pauline Zaenker and Melanie R. Ziman

Cancer Epidemiol Biomarkers Prev  Published OnlineFirst September 20, 2013.

10.1158/1055-9965.EPI-13-0621

Access the most recent version of this article at:

doi:10.1158/1055-9965.EPI-13-0621

Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.