

Reimbursement Policy

Serum Tumor Markers for Malignancies

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I. Policy Description

Circulating tumor biomarkers are substances detected in the blood, urine, or other body fluids that are either produced by a tumor itself or in response to its presence. These biomarkers can be used to help detect, diagnose, stage, and manage some types of cancer, because their amounts are typically elevated in individuals harboring a tumor (Hottinger & Hormigo, 2011; NCI, 2021). There are currently dozens of tumor markers in common use; this laboratory policy addresses tumor markers which may be measured in an individual's serum.

Terms such as male and female are used when necessary to refer to sex assigned at birth.

The following management of serum tumor markers is built from recommendations from the National Comprehensive Cancer Network (NCCN) Biomarkers Compendium®, which contains information “designed to support decision making around the use of biomarker testing in patients with cancer. The NCCN Biomarkers Compendium® is updated in conjunction with the NCCN Guidelines on a continual basis” (NCCN, 2023a).

II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the “Applicable State and Federal Regulations” section of this policy document.

Note: Except for where otherwise specified in the coverage criteria below, quarterly measurement of designated serum tumor markers is permitted for follow-up, monitoring, and/or surveillance

- 1) Measurement of the following serum tumor markers **MEETS COVERAGE CRITERIA** for the following indications:

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Serum Tumor Marker	Indication
Alkaline phosphatase (ALP)	<p>Bone neoplasms: workup; during treatment; surveillance</p> <p>Systemic light chain amyloidosis: initial diagnostic workup</p>
Alpha fetoprotein (AFP)	<p>Hepatocellular carcinoma: screening; workup for confirmed HCC; surveillance (every 3-6 months for 2 years, then every 6 months)</p> <p>Intrahepatic cholangiocarcinoma: workup for isolated intrahepatic mass</p> <p>Occult primary: additional workup for localized adenocarcinoma or carcinoma not otherwise specified; liver, mediastinum, or retroperitoneal mass</p> <p>Ovarian cancer/fallopian tube cancer/primary peritoneal cancer: initial workup; during primary chemotherapy; monitoring/follow-up for complete response (as clinically indicated)</p> <p>Ovarian cancers (less common):</p> <ul style="list-style-type: none"> – Borderline epithelial tumors: monitoring/follow-up (every visit if initially elevated) – Malignant germ cell tumors: surveillance (no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5) – Malignant sex cord stromal tumors: surveillance if clinically indicated. If done, frequency based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease) <p>Testicular cancer – nonseminoma: post-diagnostic workup; risk classification; surveillance (no more than every 2 months)</p> <p>Testicular cancer - pure seminoma: initial diagnostic workup; post-diagnostic workup; risk classification; post-treatment surveillance (no more than every 2 months)</p> <p>Thymomas and thymic carcinomas: initial evaluation, if appropriate</p>
Beta-2 microglobulin (B2M)	<p>B-cell lymphomas (Castleman disease; diffuse large B-cell; follicular [grade 1-2]; HIV-related; lymphoblastic; mantle cell): workup</p> <p>Chronic lymphocytic leukemia/small lymphocytic lymphoma: workup</p> <p>Multiple myeloma: initial diagnostic workup; follow-up/surveillance (as needed) for solitary plasmacytoma or solitary plasmacytoma with minimal marrow involvement</p> <p>Systemic light chain amyloidosis: initial diagnostic workup</p> <p>Waldenström macroglobulinemia / lymphoplasmacytic lymphoma: workup</p>

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Serum Tumor Marker	Indication
Beta human chorionic gonadotropin (beta-HCG)	<p>Gestational trophoblastic neoplasia: initial workup; during and post treatment (no more than weekly); follow-up/surveillance (no more than monthly for 12 months)</p> <hr/> <p>Occult primary: additional workup for localized adenocarcinoma or carcinoma not otherwise specified; individuals < 65 years of age with testes presenting with retroperitoneal mass</p> <hr/> <p>Ovarian cancer/fallopian tube cancer/primary peritoneal cancer: initial workup; during primary chemotherapy; monitoring/follow-up for complete response (as clinically indicated)</p> <hr/> <p>Ovarian cancers (less common):</p> <ul style="list-style-type: none"> – Borderline epithelial tumors: monitoring/follow-up (every visit if initially elevated) – Malignant germ cell tumors: surveillance (no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5) – Malignant sex cord stromal tumors: surveillance if clinically indicated. If done, frequency based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease) <hr/> <p>Testicular cancer – nonseminoma: post-diagnostic workup; risk classification; surveillance (no more than every 2 months)</p> <hr/> <p>Testicular cancer - pure seminoma: initial diagnostic workup; post-diagnostic workup; risk classification; post-treatment surveillance (no more than every 2 months)</p> <hr/> <p>Thymomas and thymic carcinomas: initial evaluation, if appropriate</p>
BNP or NT-proBNP	<p>Multiple myeloma: initial diagnostic workup</p> <hr/> <p>Systemic light chain amyloidosis: initial diagnostic workup</p>
Calcitonin (CALCA)	<p>Medullary carcinoma: additional workup; post-surgical evaluation; monitoring; surveillance (2-3 months postoperative, then every 6-12 months)</p> <hr/> <p>Multiple endocrine neoplasia, type 2: at diagnosis (clinical evaluation) for medullary thyroid cancer</p>
Cancer antigen 15-3 and 27.29 (CA 15-3 and 27.29)	<p>Breast cancer (invasive): monitoring metastatic disease</p>

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Serum Tumor Marker	Indication
Cancer antigen 19-9 (CA 19-9)	<p>Ampullary adenocarcinoma: workup; surveillance (every 3-6 months for 2 years, then every 6-12 months for up to 5 years as clinically indicated) for resected ampullary cancer, stage I-III</p> <hr/> <p>Appendiceal adenocarcinoma: workup to establish baseline. Abnormal measurements should be trended</p> <hr/> <p>Extrahepatic cholangiocarcinoma: workup to establish baseline; monitoring</p> <hr/> <p>Gallbladder cancer: workup to establish baseline; monitoring; surveillance (as clinically indicated), post-resection</p> <hr/> <p>Intrahepatic cholangiocarcinoma: workup to establish baseline; monitoring</p> <hr/> <p>Ovarian cancer/fallopian tube cancer/primary peritoneal cancer: initial workup; during primary chemotherapy; monitoring/follow-up for complete response (as clinically indicated)</p> <hr/> <p>Ovarian cancers (less common):</p> <ul style="list-style-type: none"> – Borderline epithelial tumors: monitoring/follow-up (every visit if initially elevated) – Malignant germ cell tumors: surveillance (no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5) – Malignant sex cord stromal tumors: surveillance if clinically indicated. If done, frequency based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease) – Mucinous carcinoma of the ovary: additional workup (if not previously done) <hr/> <p>Pancreatic adenocarcinoma: workup to establish baseline; monitoring; post-operative, post-adjuvant treatment surveillance (every 3-6 months for 2 years, then every 6-12 months as clinically indicated)</p> <hr/> <p>Small bowel adenocarcinoma: workup to establish baseline; post-treatment surveillance (every 3-6 months for 2 years, then every 6 months for a total of 5 years)</p>
Cancer antigen 125 (CA-125)	<p>Appendiceal adenocarcinoma: workup to establish baseline</p> <hr/> <p>Endometrial carcinoma: additional workup; surveillance (if initially elevated)</p> <hr/> <p>Lynch syndrome: surveillance</p> <hr/> <p>Occult primary: additional workup for adenocarcinoma or carcinoma not otherwise specified, in those with a uterus and/or ovaries present</p>

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Serum Tumor Marker	Indication
	<p>Ovarian cancer/fallopian tube cancer/primary peritoneal cancer: initial workup; during primary chemotherapy; monitoring/follow-up for complete response (as clinically indicated)</p> <hr/> <p>Ovarian cancers (less common):</p> <ul style="list-style-type: none"> – Borderline epithelial tumors: monitoring/follow-up (every visit if initially elevated) – Malignant germ cell tumors: surveillance (no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5) – Malignant sex cord stromal tumors: surveillance if clinically indicated. If done, frequency based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease) <hr/> <p>Peritoneal mesothelioma: initial evaluation</p>
<p>Carcinoembryonic antigen (CEA)</p>	<p>Appendiceal adenocarcinoma: workup to establish baseline; monitoring; post-treatment surveillance</p> <hr/> <p>Breast cancer (invasive): Monitoring metastatic disease</p> <hr/> <p>Colon cancer: workup to establish baseline; monitoring; surveillance (every 3-6 months for 2 years, then every 6 months for a total of 5 years)</p> <hr/> <p>Extrahepatic cholangiocarcinoma: workup to establish baseline; monitoring</p> <hr/> <p>Gallbladder cancer: workup to establish baseline; monitoring; surveillance (as clinically indicated), post-resection</p> <hr/> <p>Intrahepatic cholangiocarcinoma: workup to establish baseline; monitoring</p> <hr/> <p>Medullary carcinoma: diagnosis and additional workup; monitoring; post-surgical surveillance (2-3 months postoperative, then every 6-12 months)</p> <hr/> <p>Multiple endocrine neoplasia, type 2: at diagnosis (clinical evaluation) for medullary thyroid cancer</p> <hr/> <p>Ovarian cancer/fallopian tube cancer/primary peritoneal cancer: initial workup; during primary chemotherapy; monitoring/follow-up for complete response (as clinically indicated)</p> <hr/> <p>Ovarian cancers (less common):</p> <ul style="list-style-type: none"> – Borderline epithelial tumors: monitoring/follow-up (every visit if initially elevated)

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Serum Tumor Marker	Indication
	<ul style="list-style-type: none"> – Malignant germ cell tumors: surveillance (no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5) – Malignant sex cord stromal tumors: surveillance if clinically indicated. If done, frequency based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease) – Mucinous carcinoma of the ovary: additional workup (if not previously done) <hr/> <p>Rectal cancer: workup to establish baseline; monitoring; surveillance (every 3-6 months for 2 years, then every 6 months for a total of 5 years)</p> <hr/> <p>Small bowel adenocarcinoma: workup to establish baseline; post-treatment surveillance (every 3-6 months for 2 years, then every 6 months for a total of 5 years)</p>
Inhibin (INHA)	<p>Ovarian cancer/fallopian tube cancer/primary peritoneal cancer: initial workup; during primary chemotherapy; monitoring/follow-up for complete response (as clinically indicated)</p> <hr/> <p>Ovarian cancers (less common):</p> <ul style="list-style-type: none"> – Borderline epithelial tumors: monitoring/follow-up (every visit if initially elevated) – Malignant Germ cell tumors: surveillance (no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5) – Malignant sex cord stromal tumors: surveillance if clinically indicated. If done, frequency based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease)
Lactate dehydrogenase (LDH)	<p>B-cell lymphomas (Burkitt; Castleman disease; diffuse large B-cell; extranodal marginal zone lymphoma of nongastric sites [noncutaneous] and of the stomach; follicular [grade 1-2]; HIV-related; lymphoblastic; mantle cell; nodal marginal zone; pediatric aggressive mature; post-transplant lymphoproliferative disorders; primary cutaneous; splenic marginal zone): workup</p> <hr/> <p>Bone neoplasms: workup</p> <hr/> <p>Chronic lymphocytic leukemia/small lymphocytic lymphoma: workup, and at transformation or histologic progression (if applicable)</p> <hr/> <p>Hairy cell leukemia: workup</p> <hr/> <p>Kidney cancer: initial workup</p>

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Serum Tumor Marker	Indication
	<p>Melanoma (cutaneous and uveal): workup for metastatic or recurrent disease</p> <hr/> <p>Multiple myeloma: initial workup; surveillance (as needed) post primary treatment for solitary plasmacytoma or solitary plasmacytoma with minimal marrow involvement</p> <hr/> <p>Ovarian cancer/fallopian tube cancer/primary peritoneal cancer: initial workup; during primary chemotherapy, monitoring/follow-up for complete response (as clinically indicated)</p> <hr/> <p>Ovarian cancers (less common):</p> <ul style="list-style-type: none"> – Borderline epithelial tumors: monitoring/follow-up (every visit if initially elevated) – Malignant germ cell tumors: surveillance (no more than every 2 months for the first 2 years, every 4 months in years 3-5, and then annually after year 5) – Malignant sex cord stromal tumors: surveillance if clinically indicated. If done, frequency based on stage (i.e., 6-12 months if early-stage, low-risk disease; 4-6 months if high-risk disease) <hr/> <p>Primary cutaneous lymphomas (mycosis fungoides/Sezary syndrome; primary cutaneous CD30+ T-cell lymphoproliferative disorders): workup</p> <hr/> <p>Systemic light chain amyloidosis: initial diagnostic workup</p> <hr/> <p>T-cell lymphomas (adult T-cell; breast implant-associated ALCL; extranodal NK/T-cell; hepatosplenic; peripheral; T-cell prolymphocytic leukemia): workup; staging (breast implant-associated ALCL only)</p> <hr/> <p>Testicular cancer – nonseminoma: post-diagnostic workup; risk classification; surveillance (no more than every 2 months)</p> <hr/> <p>Testicular cancer – pure seminoma: initial diagnostic workup; post-diagnostic workup; risk classification; post-treatment surveillance (no more than every 2 months)</p> <hr/> <p>Waldenström macroglobulinemia / lymphoplasmacytic lymphoma: workup</p>
Serum free light chain	<p>Multiple myeloma: initial diagnostic workup; surveillance (up to once per month)</p> <hr/> <p>Systemic light chain amyloidosis: initial diagnostic workup</p>
Troponin T	<p>Systemic light chain amyloidosis: initial diagnostic workup</p>
Tryptase	<p>Systemic mastocytosis: initial diagnosis</p>

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The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness:

- 2) For all other cancer indications not discussed above, use of the above biomarkers (alone or in a panel of serum tumor markers) **DOES NOT MEET COVERAGE CRITERIA.**
- 3) All other serum tumor markers not addressed above (alone or in a panel of serum tumor markers) **DO NOT MEET COVERAGE CRITERIA.**
- 4) For the screening and detection of cancer, analysis of proteomic patterns in serum **DOES NOT MEET COVERAGE CRITERIA.**

III. Table of Terminology

Term	Definition
A2-PAG	Pregnancy associated alpha 2 glycoprotein
AACC	American Association for Clinical Chemistry
AASLD	American Association for the Study of Liver Diseases
ACCP	American College of Chest Physicians
ACR	American College of Radiology
AFP	Alpha fetoprotein
AGCT	Adult-type granulosa cell tumor
AIDS	Acquired immune deficiency syndrome
ALL	Acute lymphoblastic leukemia
ALP	Alkaline phosphatase
AMH	Anti-müllerian hormone
AML	Acute myeloid leukemia
ASCO	American Society of Clinical Oncology
ATA	American Thyroid Association
AUC	Area under curve
B7-H4	V-set domain-containing T-cell activation inhibitor 1
B2M	Beta-2 microglobulin
BCM	Breast cancer mucin
beta-HCG	Beta human chorionic gonadotropin
BNP	Brain natriuretic peptide
BRCA	Breast cancer gene
<i>BRCA1</i>	<i>Breast cancer gene 1</i>
<i>BRCA2</i>	<i>Breast cancer gene 2</i>

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Term	Definition
CA	Cancer antigen
CALCA	Calcitonin
CAM 17-1	Antimucin monoclonal antibody
CAM-26	Carcinoma associated mucin antigen
CAM-29	Carcinoma associated mucin antigen
CAR-3	Antigenic determinant recognized by monoclonal antibody AR-3
CA-SCC	Squamous cell carcinoma antigen
CEA	Carcinoembryonic antigen
CEACAM6	Carcinoembryonic antigen cell adhesion molecule 6
CEACAM-7	Carcinoembryonic antigen cellular adhesion molecule-7
CEP17	Chromosome 17 centromere
CFL1	Cofilin
CgA	Chromogranin A
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CRC	Colorectal cancer
CSS	Cancer specific survival
CTC	Circulating tumor cell
CUP	Cancers of unknown primary
<i>CYP2D6</i>	<i>Cytochrome P450 2D6</i>
DCIS	Ductal carcinoma in situ
DCP	Des- γ -carboxy prothrombin
DcR3	Decoy receptor 3
DFS	Disease-free survival
DMSA	Pentavalent technetium-99mm dimercaptosuccinic
Du-PAN-2	Sialylated carbohydrate antigen
EASL	European Association for the Study of the Liver
ECM	Extracellular matrix protein
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay
EPCAM	Epithelial cell adhesion molecule
ER	Estrogen receptor
FDA	Food and Drug Administration
FLC	Free-light chain
FOXP3	Forkhead box P3
GC	Gastric cancer
GCTs	Germ cell tumors

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Term	Definition
GRP78	78-kDa glucose-regulated protein
HCC	Hepatocellular carcinoma
HE4	Human epididymis protein 4
HEC1	Highly expressed in cancer protein
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratio
HYAL1	Hyaluronoglucosaminidase
IGF	Insulin-like growth factors
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
INHA	Inhibin
Ki-67	Antigen KI-67
KRAS	Kirsten rat sarcoma viral oncogene homolog
LCA	Lens culinaris agglutinin
LCOC	Less common ovarian cancers
LCOH	Less common ovarian histopathologies
LDH	Lactate dehydrogenase
LDT	Laboratory-developed test
LINE-1	Long interspersed nuclear elements 1
MALDI	Matrix-assisted laser desorption/ionization
MAP	Microtubule-associated protein
MCA	Mucinous carcinoma associated antigen
MGUS	Monoclonal gammopathy of undetermined significance
MHC	Major histocompatibility complex
MINDACT	Microarray in node-negative disease may avoid chemotherapy
MMP-1	Matrix metalloproteinase-1
mRNA	Messenger ribonucleic acid
MSA	Mammary serum antigen
MTC	Medullary thyroid carcinoma
NACB	National Academy of Clinical Biochemistry
NANETS	North American Neuroendocrine Tumor Society
NCCN	National Comprehensive Cancer Network
NET	Neuroendocrine tumor cells
NICE	National Institute for Health and Clinical Excellence
NMP22	Nuclear matrix protein 22
non-HCC	Non-hepatocellular carcinoma

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Term	Definition
NSE	Neuron specific enolase
NSGCT	Nonseminomatous germ cell tumor
NT-proBNP	N-terminal-pro hormone brain natriuretic peptide
OS	Overall survival
P53	Tumor protein P53
PAGE	Polyacrylamide gel electrophoresis
PAI-1	Plasminogen activator inhibitor type 1
PAM50-ROR	Prediction analysis of microarray 50-risk of recurrence
PcSt	Pancreastatin
PD-L1	Programmed Death-ligand 1
PED-ALL	Pediatric acute lymphoblastic leukemia
PgR	Plant growth regulator
PIVKA-II	Protein induced by vitamin K absence/antagonist-II
P-LAP	Placental alkaline phosphatase
PNA-ELLA	Peanut lectin bonding assay
PR	Progesterone receptor
PSA	Prostate specific antigen
PTEN	Phosphatase and tensin homolog
RCC	Renal cell carcinoma
RMI I	Risk of malignancy index I
ROC	Receiver operating characteristic
ROMA	Risk of ovarian malignancy algorithm
ROR	Risk of recurrence
RRSO	Risk-reducing salpingo-oophorectomy
SCC	Squamous cell carcinoma
SCLCs	Small cell lung cancers
SLEX	Sialylated lewis-x antigen
SLX	Sialylated SSEA-1 antigen
SPAN-1	Sialylated carbonated antigen span-1
ST-439	Sialylated carbohydrate antigen st-439
STMs	Serum tumor markers
TAG	Tumor associated glycoprotein
TATI	Tumor associated trypsin inhibitor
TILs	Tumor-infiltrating lymphocytes
TIMP-1	Tissue inhibitor of metalloproteinase-1
TKI	Tyrosine kinase inhibitor
TN	Triple-negative
TNF-a	Tumor necrosis factor alpha

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Term	Definition
TOP2A	Deoxyribonucleic acid topoisomerase II alpha
TPA	Tissue polypeptide antigen
TPS	Tissue polypeptide specific antigen
TTF-1	Thyroid transcription factor-1
TVUS	Transvaginal ultrasound
uPA	Urokinase plasminogen activator
uPAR	Urokinase plasminogen activator receptor
WT1	Wilms' tumor protein

IV. Scientific Background

Actionable molecular assays for tumor biomarkers may guide treatment decisions for common malignancies (Febbo et al., 2011). Tumor biomarkers are proteins detected in blood, urine, or body fluids that serve as surrogate indicators to increase or decrease the clinician's suspicion of future clinically important events. These can be used to determine risk, screen for early cancers, establish diagnosis, estimate prognosis, predict that a specific therapy will work, and/or monitor for disease recurrence or progression (Catharine M. Sturgeon et al., 2008). The National Comprehensive Cancer Network (NCCN) task force guidelines recommend that tumor markers be classified by indication as diagnostic, prognostic, predictive, and companion tests. An individual marker may serve more than one purpose and thus can fall into more than one category of biomarker. Biomarkers may also have different categorization across different stages of disease or different types of tumors (Febbo et al., 2011). Some of these categories are listed below:

- Diagnostic biomarkers – Tumor biomarkers that aid in the diagnosis or subclassification of a particular disease state. Detection of diagnostic biomarkers may result in different management of the disease, but the marker is used primarily to establish that a particular disease is present in the patient sample. An example of a diagnostic biomarker is the Philadelphia chromosome in chronic myelogenous leukemia.
- Prognostic – Tumor biomarkers that have an association with some clinical outcomes, such as overall survival or recurrence-free survival, independent of the treatment rendered. An example is the p53 gene, whose presence may indicate a more aggressive type of cancer.
- Predictive - Tumor biomarkers predict the activity of a specific class or type of therapy and are used to help make more specific treatment decisions. An example is human epidermal growth factor 2 (HER2), which is assessed in breast cancer patients. Patients who are negative for this biomarker do not respond as well to trastuzumab.
- Companion - Biomarkers may be diagnostic, prognostic, or predictive, but are used to identify a subgroup of patients for whom a therapy has shown benefit. This category of

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biomarker is similar to the predictive category, but these biomarkers do not usually have independent prognostic or predictive strength (Febbo et al., 2011).

Proprietary Testing

There are laboratory developed tests for serum tumor markers, but the clinical validity of these test have not been clearly proven yet.

The IMMray® PanCan-d test measures nine serum biomarkers, including immunoregulatory and tumor biomarkers. The test uses IMMray microarray technology that prints single chain fragment antibodies onto a slide. The slide is preloaded with a microarray of antibodies. The slide can then be screeded to measure the serum response to each biomarker. In a blind validation study, the test was reported to have a 99% specificity and 89% sensitivity rate when identifying stages I and II PDAC (n=56) versus high-risk individual controls. The accuracy increased to 92% sensitivity and 99% specificity across stage I-IV PDAC (n=157) (Immunovia, 2023).

BeScreened™-CRC is a colorectal cancer screening test. BeScreened™-CRC tests three blood-based proteins that are connected to the immunological activities of colorectal cancer. The test results are reported as either “negative” or “positive” for the likely presence of CRC. The test is reported to have 94% accuracy in determining the “likely presence or absence of colorectal cancer.” “BeScreened™-CRC is not a test for colorectal cancer diagnosis; it is a screening test that aides in the detection of colorectal cancer and is not intended to replace a colonoscopy” (BeScreened, 2023).

REVEAL Lung Nodule Characterization is a blood test that specifically aids in “characterizing indeterminate pulmonary nodules (4-30mm) in current smokers aged 25 years and older.” The test results are based on three clinical factors and three blood proteins associated with lung cancer. “REVEAL Lung Nodule Characterization is a risk assessment tool, that is to be used only in conjunction with standard clinical assessments. The test is not intended as a screening or stand-alone diagnostic assay” (MagArray, 2023).

OVA1® and OVERA® are blood tests for ovarian cancer. OVA1® has FDA-clearance for testing ovarian cancer risk in women planning to have surgery for a pelvic mas. OVA1® is a first-generation multivariate index assay that measures five ovarian cancer-associated markers. “A negative OVA1 (MIA) result is accompanied by a 98% likelihood that the woman being tested is in fact disease-free.” OVERA® has been FDA-cleared for people with a pelvic mass who are planned for surgery. OVERA® is a second-generation multivariate index assay that measures different markers and uses a more refined algorithm than OVA1® (ASPIRE, 2023).

Clinical Utility and Validity

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Most biomarkers are not specific for tumors or organs, and their levels may rise in other diseases. The diagnostic value of a tumor marker will depend on the prevalence of the disease and on the specificity and sensitivity of the marker (Hottinger & Hormigo, 2011). The analytic and clinical validity as well as the clinical utility of each biomarker should be taken into account before its use for screening and or management of malignancies (Catharine M. Sturgeon et al., 2008). Establishing a biomarker's ability to associate with a given outcome of interest (diagnostic, prognostic, et al.) and ability to improve clinical outcomes and decision-making is critical (Febbo et al., 2011).

With respect to biomarker acquisition, growing evidence continues to support the utility of liquid biopsy. Compared to the “gold standard” tissue biopsy, serum can be obtained in a relatively non-invasive manner, without the need for surgery and the associated risks and recovery time. Further, serum is generally always available; tumor tissue, conversely, may not always be accessible or present in a clinically useful quantity (Pinzani et al., 2021).

Alpha-fetoprotein (AFP)

Alpha-fetoprotein (AFP) is a commonly assessed biomarker in cancer patients. AFP is a protein that is normally produced by the fetal yolk sac, and its concentration stabilizes at approximately $< 10 \mu\text{g/L}$ shortly after birth (Schefer et al., 1998). Many tissues produce this protein if they become malignant, and AFP is elevated in a variety of cancers, such as hepatocellular carcinomas. False positives may occur due to liver damage or a rare hereditary syndrome (Gilligan et al., 2010).

Alpha-fetoprotein can be fractionated into three different isoforms based on reactivity with Lens culinaris agglutinin (LCA), and the three types are as follows: L1 (no reactivity), L2 (low reactivity), L3 (high reactivity). AFP-L3 is theorized to associate with HCC because the dedifferentiation of HCC tissues correlates with the production of the enzyme that produces AFP-L3. This means that AFP-L3 may be closely related to cancer-specific events and are at least more specific to more malignant cancers (M. Wu et al., 2018).

A study by Santos Schraiber et al. (2016) assessed the ability to predict recurrence of hepatocellular carcinoma (HCC) after liver transplant using AFP. The authors analyzed 206 patients, and the recurrence frequency was found to be 15.5%. However, the authors' multivariate analysis found that the only risk factor for recurrence was an AFP level of $>200 \text{ ng/mL}$, which was associated with a 3.32 times higher increase in the probability of HCC recurrence. The authors noted that recurrence was also associated with lower survival rate (Santos Schraiber et al., 2016).

Cheng et al. (2014) conducted a meta-analysis of fifteen studies (4465 patients) to evaluate the association between high pre-treatment serum AFP-L3% and overall survival (OS) and disease-

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free survival (DFS) in HCC patients. The authors found that high pre-treatment serum AFP-L3% implied poor OS (Hazard Ratio [HR]: 1.65, and DFS (HR: 1.80) of HCC. The authors found an association between pre-treatment serum AFP-L3% and OS and DFS in low AFP concentration HCC patients (HR: 1.96 and 2.53 respectively). The authors concluded that “high pre-treatment serum AFP-L3% levels indicated a poor prognosis for patients with HCC” (Cheng et al., 2014).

Park et al. (2017) compared the diagnostic values of AFP, AFP-L3, and PIVKA-II individually and in combination to find the best biomarker or biomarker panel. A total of 79 patients with newly diagnosed HCC and 77 control patients with liver cirrhosis were enrolled. When the three biomarkers were analyzed individually, AFP showed the largest area under the receiver-operating characteristic curve (AUC) (0.751). For combinations of the biomarkers, the AUC was highest (0.765) for PIVKA-II > 40 mAU/mL and AFP > 10 ng/mL. Adding AFP-L3 > 10% led to worse sensitivity and lower AUC. The authors concluded that “the diagnostic value of AFP was improved by combining it with PIVKA-II, but adding AFP-L3 did not contribute to the ability to distinguish between HCC and non-HCC liver cirrhosis” and that “AFP showed the best diagnostic performance as a single biomarker for HCC” (Park et al., 2017).

Ryu et al. (2017) investigated the prognostic implications of the expression patterns of three tumor markers, alpha-fetoprotein (AFP), the Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) and des- γ -carboxy prothrombin (DCP). The study included 1182 consecutive patients that underwent hepatic resection and surgical microwave ablation for HCC. This study analyzed 475 patients within the Milan criteria and Child-Pugh class A. Cumulative overall survival (OS) and disease-free survival (DFS) rates were analyzed relative to the number of positive tumor markers. OS and DFS at five years postoperatively were 85.3 and 44.2% in triple-negative patients, 79.4 and 48.0% in single-positive patients, 56.2 and 32.9% in double-positive patients, and 61.7 and 35.7% in triple-positive patients. OS in triple-negative or single-positive patients was 85.3%, and that in all double- or triple-positive patients was 58.0%; DFS at five years postoperatively in these two groups was 45.9 and 34.0%, respectively. The authors concluded that “both double- and triple-positive tumor markers are associated with early recurrence and poor survival in HCC patients within the Milan criteria and Child-Pugh class A” (Ryu et al., 2017).

Caviglia et al. (2016) conducted a study evaluating AFP, AFP-L3, and DCP as detection tools for HCC. A total of 98 patients were enrolled (44 without HCC, 54 with), and the FDA-approved uTASWako was used to measure these biomarkers. AFP-L3 had an area under the curve of 0.867, a sensitivity of 0.849, a specificity of 0.886, a negative predictive value of 0.830, and a positive predictive value of 0.900. The combination of all three biomarkers had an accuracy of 87.6%. The overall accuracy of uTASWako was 84.5%. The authors concluded that the uTASWako had

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a “high analytical performance” and that the biomarker combination was superior to any of them alone (Caviglia et al., 2016).

Beta-human chorionic gonadotropin (beta-hCG)

Beta-human chorionic gonadotropin (beta-hCG) is the beta subunit of the normal hCG hormone produced during pregnancy. Some malignancies express the gene for the beta subunit of hCG, thereby producing this protein outside of pregnancy (Harvey, 2023). The beta subunit is responsible for providing the biological and immunological specificity to each hormone (Marcillac et al., 1992). This biomarker is typically associated with aggressive disease in nontrophoblastic tumors. This biomarker may be seen in ovarian cancers, testicular cancers, and more (Hotakainen et al., 2002).

Li et al. (2018) evaluated beta-hCG as a marker for colorectal cancer (CRC). In total, 50 patients out of 136 patients expressed beta-hCG at the “invasive front.” The authors found that higher expression of beta-hCG to be associated with worse prognosis than those with low beta-hCG expression and that beta-hCG “promoted the migration and invasion of CRC in vitro and in vivo but had no effect on the proliferation of tumor cells.” A correlation was also found between beta-hCG expression level and tumor invasion in early-stage CRC patients (Li et al., 2018).

Beta-2 microglobulin (B2M)

Beta-2 microglobulin (B2M) is the light chain component of the MHC-1 molecule and is present in most cells of the body (Berrebi et al., 2009). This protein may aggregate and eventually form insoluble amyloid fibrils, which cause numerous conditions such as bone and joint damage (Katou et al., 2002; Marcinko et al., 2017). Elevated serum levels of B2M have been associated with cancers such as multiple myeloma or chronic leukocytic leukemia (Berrebi et al., 2009).

Seo et al. (2016) examined the prognostic value of B2M for diffuse large B-cell lymphoma. A total of 833 patients at a ≥ 2.5 mg/L cutoff were analyzed, and both five-year survival and overall survival rates were found to be significantly worse in patients with elevated B2M (290 patients or 34.8%). The elevated B2M cohort was calculated to have a 41% five-year survival rate and a 49.2% overall survival rate, compared to 76.1% five-year survival and 83.8% overall survival for the remaining 543 patients (Seo et al., 2016).

Calcitonin

Serum calcitonin is the primary tumor marker for medullary thyroid carcinoma (MTC). MTC is a neuroendocrine tumor of the parafollicular or C cells of the thyroid gland, and production of calcitonin is a signifying characteristic of this tumor. The concentration of calcitonin tends to

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correlate with tumor mass (Tuttle, 2023). However, the ATA has noted this biomarker to have significant uncertainties (Haugen et al., 2016; Wells et al., 2015).

Tormey et al. (2017) evaluated measurement of serum calcitonin in patients presenting with thyroid nodules. A total of 44 patients were evaluated, and 33 of the patients did not have a “detectable serum calcitonin,” noting that three patients had an initially elevated serum concentration that became undetectable. The authors also note that out of the 2070 patients in their sample, only seven cases of medullary thyroid cancer (MTC) were diagnosed. The authors recommended not screening routinely for MTC (Tormey et al., 2017).

Cancer antigens (CA)

Cancer antigens (CA) refer to any substance produced by the body in response to a tumor. Various cancer antigens have been proposed as biomarkers for numerous types of cancer, such as CA 19-9, CA 125, and CA 15-3. CA 19-9 (also called carbohydrate antigen) refers to a specific antibody that binds a sialyl compound produced by cancer tissue (Sialyl Lewis A). CA 19-9 is elevated in several different types of cancer, such as adenocarcinomas or colorectal cancer (Magnani, 2004). CA 125 is a glycoprotein produced in fetal tissue as well as mesothelial cells in adults (Isaksson et al., 2017). Its function is thought to assist with cell adhesion, metastasis, and immunosuppression (Dorigo & Berek, 2011).

Kim et al. (2017) performed a study assessing the association of serum CA 19-9 and CEA with colorectal neoplasia. A total of 124509 measurements of serum CEA level and 115833 measurements of serum CA 19-9 were taken. All subjects were asymptomatic and underwent a colonoscopy. Elevated serum levels of CEA were found to be associated with any adenoma. Elevated CA 19-9 was found to be associated with high-risk or advanced adenoma, CRC, and advanced colorectal neoplasia (Kim et al., 2017).

A study was performed by Feng et al. (2017) that focused on the diagnostic and prognostic value of CEA, CA 19-9, AFP, and CA125 for early gastric cancer. The authors evaluated 587 patients, and the positive rate for all markers combined was 10.4%. CEA’s positive rate was 4.3%, CA 19-9’s was 4.8%, AFP’s was 1.5%, and CA125’s was 1.9%. The authors noted that elevated CEA was correlated with lymph node metastasis and concluded that CEA was an independent risk factor for poor prognosis of early gastric cancer (Feng et al., 2017).

Lucarelli et al. (2014) evaluated CA 15-3, CA125, and B2M as biomarkers for renal cell carcinoma (RCC). A total of 332 patients undergoing nephrectomy for RCC were analyzed. The authors found that 35.2% (117/332) of patients had abnormal levels of CA 15-3, 9.6% (32/332) had abnormal levels of CA125, and 30.4% (101/332) had abnormal B2M. Cancer specific survival (CSS) rates significantly decreased for high levels of any of the three biomarkers, and

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at a multivariate analysis found high levels of CA 15-3 to be an independent adverse prognostic risk factor for CSS (Lucarelli et al., 2014).

Chen et al. (2018) analyzed four serum tumor markers in patients with ovarian tumors. HE4, CA-125, CA19-9, and CEA were all studied. The authors evaluated 386 healthy controls, 262 patients with benign ovarian tumors, and 196 patients with malignant ovarian tumors. The authors found that the serum marker levels were significantly higher in patients with malignant tumors than the two other groups. HE4 was found to have a high specificity (96.56%) in malignant tumors. HE4, CA125, CA19-9, and CEA had sensitivities of 63.78%, 62.75%, 35.71%, and 38.78%, respectively. HE4 and CA125 combined was found to have the highest diagnostic sensitivity at 80.10%, as well as a specificity of 69.08%. Although adding markers to the HE4-CA125 combination increased diagnostic sensitivity (to 88.52%), this difference was not considered significant (Chen et al., 2018).

Isaksson et al. (2017) performed a study of tumor markers' association with resectable lung adenocarcinomas. The study evaluated blood samples from 107 patients with stages I-III lung adenocarcinoma and examined the following markers: CEA, CA 19-9, CA 125, human epididymis protein 4 (HE4), and neuron-specific enolase (NSE). When the authors calculated the disease-free survival rate, CA 19-9 and CA 125 were found to be significantly associated with recurrent disease with a combined hazard ratio of 2.8. The authors stated that "high pre-operative serum CA 19-9 and/or CA 125 might indicate an increased incidence of recurrent disease in resectable lung adenocarcinomas" (Isaksson et al., 2017).

Bind et al. (2021) evaluated the diagnostic ability of CA19-9 and CA 125 for gallbladder cancers. A total of 118 patients were included, 91 benign cases and 27 malignant. The mean value of CA19-9 was found to be 12.86 U/mL in benign cases and 625.35 U/mL in malignant cases. For CA 125, the mean value for benign cases was found to be 17.98 U/mL, and for malignant cases, 239.63 U/mL. The authors examined a theoretical diagnostic cut-off value of 252.31 U/mL for CA19-9 and 92.19 U/mL for CA 125. At this cutoff, sensitivity and specificity for CA19-9 was 100% and 98.9% respectively, and for CA 125, 100% and 94.5%. The authors concluded that "...both serum CA 19-9 and serum CA 125 may act as a good adjunct for diagnosis of cases of carcinoma gallbladder along with imaging studies. However, changes in CA19-9 are more significant than CA 125" (Bind et al., 2021).

Carcinoembryonic antigen (CEA)

Carcinoembryonic antigen (CEA) is a protein normally produced by fetal tissue, and as with AFP, stabilizes soon after birth. CEA is often elevated in malignancies such as breast or pancreatic cancer, although other conditions such as liver damage or cigarette smoking may

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affect CEA levels as well (Ueland, 2023). The gene encoding CEA encompasses certain genes encoding for cell adhesion, as well as MHC antigens (Duffy, 2001).

Chromogranin A (CgA)

Chromogranins are proteins contained in neurosecretory vesicles of NET cells and are typically elevated in neuroendocrine neoplasms. CgA is the most sensitive of the three chromogranins, and as such as the primary marker used to evaluate neoplasms. However, this biomarker is highly variable (Strosberg, 2023).

A meta-analysis performed by Yang et al. (2015) assessed the association of CgA with neuroendocrine tumors. The analyses included 13 studies totaling 1260 patients (967 healthy controls), and the pooled sensitivity was found to be 0.73. The pooled specificity was found to be 0.95. However, the study stressed that further research needs to be undertaken (Yang et al., 2015). Another study by Tian et al. (2016) found that although median CgA levels were significantly higher than healthy controls (93.8 ng/mL compared to 37.1 ng/mL), only a weak correlation was found between changes in serum CgA levels and clinical regimen. The CgA cutoff value for this study was 46.2 ng/mL, which led to a sensitivity of 78.8% and specificity of 73.8% (Tian et al., 2016).

Lactate Dehydrogenase (LDH)

Lactate Dehydrogenase (LDH) is an enzyme that catalyzes the interconversion between lactate and pyruvate. LDH is often found to be upregulated in tumors, and a key feature of cancer sites is the accumulation of lactate or lactic acid. This is thought to be caused by increased glycolysis and this increase in lactate causes an elevated concentration of LDH (Pucino et al., 2017). Increased LDH is found in several different cancers, such as B-cell lymphomas and osteosarcomas (NCCN, 2023t).

Liu et al. (2016) performed a study evaluating the OS rates of an extremely high concentration of LDH (>1000 IU/L, considered by the study to be four times the upper normal limit). A total of 311 patients with >1000 U/L were examined, and the OS rate of this cohort was 1.7 months with 163 perishing within two months. However, 51 patients' LDH decreased to normal following chemotherapy, and the OS rate of this group was 22.6 months. The cohort who survived at two months but did not see their LDH decrease had an OS rate of four months. There was no positive association found between OS and type of cancer, although there were different OS rates for patients at different stages of lymphoma (Liu et al., 2016).

Inhibins

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The primary function of inhibins is to inhibit hormones such as follicle stimulating hormone. However, since this protein is restricted to ovarian granulosa cells in women, unusual levels of inhibins may signal tumors in this region (Walentowicz et al., 2014). This marker exists as two different isoforms, inhibin A and B. Either form can be measured, although an active tumor may over-secrete one or both forms (Gershenson, 2023). Inhibin B is generally considered to be more accurate than inhibin A, with sensitivities ranging from 0.88 to 1.00 whereas inhibin A's sensitivity ranges from 0.67-0.77. However, inhibin B has limitations of its own such as fluctuations with the menstrual cycle (Farkkila et al., 2015).

Farkkila et al. (2015) evaluated anti-Müllerian hormone (AMH) and inhibin B in the context of ovarian adult-type granulosa cell tumors (AGCTs). The study included 560 samples taken from 123 patients, and both markers were significantly elevated in AGCTs. The area under the curve for inhibin B was 0.94, but measurement of both markers was noted to be a better method than measuring either marker individually (Farkkila et al., 2015).

Urokinase plasminogen activator (uPA)

Urokinase plasminogen activator (uPA) is a serine protease with an important role in cancer invasion and metastases (Stephens et al., 1998). When bound to its receptor (uPAR), uPA converts plasminogen into plasmin and mediates degradation of the extracellular matrix during tumor cell invasion. High levels have been associated with shorter survival in women with breast cancer (Chappuis et al., 2001; Foekens et al., 2000; Malmstrom et al., 2001; Stephens et al., 1998). ASCO guidelines include the option for using uPA and PAI-1 to guide decisions on adjuvant systemic therapy for patients with node-negative, hormone-positive/HER2-negative disease, but not for patients with HER2-positive or triple-negative disease (L. N. Harris et al., 2016; Theodoros & Bergh, 2023).

Serum free light chains

Light chains are proteins produced by plasma cells that, along with heavy chains, collectively make up an immunoglobulin macromolecule. There are a total of five heavy chain protein classes (IgG, IgE, IgA, IgD, and IgM), and two light chain protein classes (kappa and lambda). Healthy plasma cells produce polyclonal immunoglobulins that are capable of binding to antigens and inducing an immune response; unhealthy plasma cells produce monoclonal immunoglobulins that do not effectively engage antigens (Kyrtsolis MC, 2012). In the case of certain plasma cell disorders, an abundance of monoclonal immunoglobulin or free light chains (kappa and/or lambda), may accumulate in the serum and serve as useful diagnostic markers.

Multiple myeloma is an uncontrolled growth of plasma cells (ACS, 2018a). In most cases, the cancerous clonal cells secrete an intact monoclonal immunoglobulin, and the gold standard for

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diagnosis is serum protein electrophoresis and immunofixation (Tosi et al., 2013). Less commonly, however, myeloma clones will secrete only light chains; in these instances, a serum free light chain assay can be employed to quantify the ratio of kappa and lambda chains in the serum. It has been demonstrated that in healthy individuals, the kappa/lambda ratio in the serum is approximately 0.58 (Katzmann et al., 2002). In the case of plasma cell neoplasms, free light chains are overproduced, and the kidneys are unable to completely clear them, resulting in accumulation in the serum and a change in the kappa/lambda ratio. This ratio is often used to aid in the diagnosis, prognosis, and monitoring of plasma cell disorders (Tosi et al., 2013).

Waldenström's Macroglobulinemia (WM) is a type of cancer that is similar to multiple myeloma and non-Hodgkin lymphoma. WM cells are called “lymphoplasmacytoid” because they have features of both plasma cells and lymphocytes (ACS, 2018b). WM cells are distinguished by the production of immunoglobulin M (IgM) serum monoclonal protein, also referred to as a “macroglobulin” (Cautha et al., 2022). While serum IgM level is useful for diagnostic purposes, it does not correlate with prognosis. The addition of a serum free light chain assay to the care of patients with suspected Waldenström's Macroglobulinemia has been shown to improve overall care, as it may help differentiate patients with another, potentially benign disorder called monoclonal gammopathy of undetermined significance (MGUS), as well as influence prognosis (Moreau AS, 2006).

Castleman disease represents a group of B-cell lymphoproliferative disorders characterized by distinct pathogenesis and clinical outcomes (Oyaert et al., 2014; D. Wu et al., 2018). Patients with suspected Castleman disease have been reported to present with abnormal levels of kappa or lambda light chains, making the serum free light chain assay a potentially useful tool in the management of this disease (Oyaert et al., 2014; D. Wu et al., 2018). Utilization of a serum free light chain assay has been shown to be clinically useful in the workup of Castleman disease, though an important caveat is that changes in the absolute values of both kappa and lambda free light chain in the serum can occur with preservation of a ratio within the normal reference range (Stankowski-Drengler et al., 2010); hence, both the free light chain ratio as well as the absolute values of each light chain protein should be considered.

Immunoglobulin light chain amyloidosis is a disorder that results from the accumulation of amyloid fibrils due to the production of fragments of monoclonal light chains (Dispenzieri, 2023; Merlini et al., 2013). As amyloid fibrils continue to accumulate, they begin to interfere with the biological function of various organs, eventually resulting in organ damage and potentially organ failure. Due to the involvement of light chains in the pathogenesis of amyloidosis, serum free light chain measurement may hold diagnostic and prognostic value, and be a viable response marker following therapy (Akar et al., 2005; Bhole et al., 2014; Kumar et al., 2010).

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Importantly, Bhole et al. (2014) highlighted key challenges with serum free light chain assays that include but are not limited to over or under-estimation of the monoclonal protein, and performance differences between available tests. Therefore, despite the demonstrated utility of these assays, clinicians should be aware of their limitations.

Proteomics

Proteomics is a qualitative and quantitative assessment of the protein constituents in a given biological sample. This is typically performed with modification of polyacrylamide gel electrophoresis (PAGE) or matrix-assisted laser desorption/ionization (MALDI). However, this method is still under investigation (Raby, 2023).

Proteomic analyses have been performed in cancer patients to assess unusual levels of protein regulation. A study by Chen et al. (2017) evaluated the proteomes of patients with CRC and healthy controls. Chen et al found 36 proteins that were upregulated in cancer patients as well as 22 proteins that were downregulated compared to healthy controls. The proteins that were upregulated tended to be processes that regulated the “pretumorigenic microenvironment for metastasis” and the downregulated proteins tended to be ones that controlled tumor growth and cell survival (Chen et al., 2017).

Qin et al. (2020) performed a “serological proteome analysis” to explore the association between an identified protein marker and gastric cancer (GC). Proteomic analysis was used to identify the protein marker of interest, an autoantibody called “anti-GRP78” (along with its corresponding antigen, the 78-kDa glucose-regulated protein [GRP78]). Two cohorts were included, a test group of 266 patients (133 GC patients, 133 controls) and a validation group of 600 patients (300 GC, 300 control). The authors found that the levels of anti-GRP78 was higher in both cohorts. The receiver operating characteristic (ROC) curve analysis found similar values for both groups to identify GC patients among control patients. The area under curve (AUC) ranged from 0.676 to 0.773 in the test group and 0.645 to 0.707 in the validation group. The authors noted this marker’s potential use as a diagnostic marker (Qin et al., 2020).

V. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

The NCCN provides a Biomarkers Compendium to “support decision-making around the use of biomarker testing in patients with cancer” (NCCN, 2023a), which serves as a primary source of guidance for coverage criteria in this policy. The Biomarkers Compendium may be accessed through [nccn.org](https://www.nccn.org).

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American Society of Clinical Oncology (ASCO)

Clinical Practice Guideline on Uses of Serum Tumor Markers (STMs) in Adult Males with Germ Cell Tumors (GCTs) were released in 2010 (Gilligan et al., 2010). ASCO recommends against any STMs to screen for GCTs. While ASCO recommends assessment of serum AFP and hCG before orchiectomy to establish a diagnosis and baseline levels, it recommends against its use to decide whether to perform an orchiectomy. The society also recommends against using these biomarkers to “guide treatment of patients with CUP and indeterminate histology.” However, substantially elevated serum AFP and/or hCG may be considered sufficient for a diagnosis in unusual cases such as patients presenting with a retroperitoneal or anterior mediastinal primary tumor. Their recommendations also include measuring serum AFP, hCG, and LDH for “all patients with testicular nonseminomatous germ cell tumors (NSGCTs) shortly after orchiectomy and before any subsequent treatment”, “before chemotherapy begins for those with mediastinal or retroperitoneal NSGCTs to stratify risk and select treatment”, and “immediately prior to chemotherapy for stage II/III testicular NSGC” (Gilligan et al., 2010).

The society recommends measuring AFP and hCG before retroperitoneal lymph node dissection in patients with stage I or II NSGCT and recommends measuring serum AFP and hCG at the start of each chemotherapy cycle and when chemotherapy concludes. These biomarkers are also recommended to be measured during surveillance after “definitive therapy for NSGCT” and this surveillance should continue for 10 years after therapy concludes (Gilligan et al., 2010).

Measuring “postorchiectomy serum concentrations of hCG and/or LDH for patients with testicular pure seminoma and preorchiectomy elevations” was also discussed, but ASCO recommends against using these concentrations for staging or prognosis. No markers are recommended to guide treatment decisions, monitor response, or progression for seminomas. However, serum hCG and AFP should be measured both when treatment concludes as well as during post-treatment surveillance. ASCO recommends these intervals: every two to four months in the first year, every three to four months in the second year, every four to six months in the third and fourth years, and annually thereafter. Surveillance should last for at least 10 years following the conclusion of therapy (Gilligan et al., 2010).

Guidelines were released on the use of biomarkers to inform treatment decisions regarding systemic therapy for women with metastatic breast cancer. “Patients with accessible, newly diagnosed metastases from primary breast cancer should be offered biopsy for confirmation of disease process and testing of ER, PR, and HER2 status. With discordance of results between primary and metastatic tissues, the panel consensus is to preferentially use the ER, PR, and HER2 status from the metastasis to direct therapy if supported by the clinical scenario and the patient’s goals for care.” Decisions on changing to a new drug or regimen, initiating, or discontinuing treatment should be based on the patient’s goals for care and clinical evaluation and judgment of disease progression or response. There is no evidence at this time that changing therapy solely based on tissue or circulating biomarker results beyond ER, PR, and

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HER2 improves health outcomes, quality of life, or cost-effectiveness. To date, clinical utility has not been demonstrated for any additional biomarkers. “CEA, CA 15-3, and CA 27.29 may be used as adjunctive assessments to contribute to decisions regarding therapy for metastatic breast cancer. Data are insufficient to recommend use of CEA, CA 15-3, and CA 27.29 alone for monitoring response to treatment” (Van Poznak et al., 2015).

A provisional clinical opinion on evaluating susceptibility to pancreatic cancer was released by ASCO, stating that “there are currently no proven biomarkers using noninvasively obtained biospecimens (eg, blood, urine, stool) for early detection of pancreatic cancer in asymptomatic individuals.” ASCO states that further validation of biomarkers is needed (Stoffel et al., 2018). Finally, a guideline on treatment of malignant pleural mesothelioma was published, stating that calretinin, keratins five and six, and nuclear WT-1 are expected to be positive while CEA, EPCAM, Claudin four, and TTF-1 should be negative. Non-tissue based biomarkers are currently not recommended due to their unvalidated statistical accuracy (Kindler et al., 2018).

Association for Diagnostics & Laboratory Medicine (ADLM); formerly the National Academy of Clinical Biochemistry (NACB) and AACC Academy

Practice guidelines on the use of tumor markers for liver, bladder, cervical, and gastric cancers were released by ADLM (Sturgeon et al., 2010). The association recommends use of AFP measurements when managing hepatocellular carcinoma (HCC). For screening, ADLM recommends AFP be measured at 6-month intervals in patients at high risk of HCC, noting that concentrations above 20 µg/L should “prompt further investigation even if an ultrasound is negative.” Sustained increases of serum AFP may be used in combination with ultrasound to inform detection and management and AFP concentrations may provide prognostic information in untreated patients. Monitoring of disease should include measurement of AFP. However, other liver biomarkers such as Glypican-3 cannot be recommended at this time without further research (Sturgeon et al., 2010).

The association did not recommend any biomarkers for the management of bladder cancer (such as NMP22, UroVysion, etc), stating that further research is required to assess their utility. ADLM did not recommend any biomarkers for screening, monitoring, prognosis, or diagnosis of cervical cancer. While pretreatment measurements of squamous cell carcinoma antigen (SCC) were acknowledged to provide information, their routine use could not be recommended. ADLM did not recommend any biomarkers for screening, diagnosis, or prognosis of gastric cancer. Routine measurement of CEA or CA 19-9 was also not recommended (Sturgeon et al., 2010).

Guidelines on use of tumor markers for testicular, prostate, colorectal, breast, and ovarian cancers were also released by ADLM (C. M. Sturgeon et al., 2008). For testicular cancer, ADLM stated that pretreatment determination of AFP, lactate dehydrogenase (LDH), and human chorionic gonadotropin (hCG) was mandatory if testicular cancer was suspected or if risk stratification and staging was done. These three biomarkers were also recommended for monitoring. ADLM notes

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that measurement of the free β -subunit of human chorionic gonadotropin (hCG β) component is essential when measuring hCG. For prostate cancer, PSA assessment is required during all stages of the disease, with ADLM recommending against age-specific intervals. PSA measuring is recommended to monitor disease status after treatment. However, ADLM did not make any recommendations on PSA screening for prostate cancer (C. M. Sturgeon et al., 2008).

For colorectal cancer (CRC), carcinoembryonic antigen (CEA) measurement is recommended every 3 months in stage II or III if “patient is a candidate for surgery or systemic therapy of metastatic disease.” Pre-operative CEA measurements may be used in conjunction with other factors to plan surgery. Regular CEA measurements should be done in patients with advanced CRC that are undergoing systemic therapy. However, CEA is not recommended for screening in healthy individuals. Routine measurement of other biomarkers such as CA 19-9, TIMP-1, or CA 242 is not recommended for prognosis or predicting response to treatment. ADLM recommends individuals older than 50 be screened for CRC. Fecal DNA is also recommended for CRC screening, as joint guidelines from other societies such as the American Cancer Society have recommended its use. Finally, ADLM supports guidelines such as the NCCN and AGA regarding genetic testing for CRC (C. M. Sturgeon et al., 2008).

According to ADLM, estrogen receptor (ER) and progesterone receptor (PR) measurements should be done in all patients diagnosed with breast cancer. HER-2 should be measured in all patients with invasive breast cancer, while urokinase plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-1) may be used to identify “lymph node–negative breast cancer patients who do not need or are unlikely to benefit from adjuvant chemotherapy.” CA 15-3, CEA, and BR 27.29 should not routinely be used for early detection in asymptomatic patients with diagnosed breast cancer. *BRCA1* and *BRCA2* mutation testing may be used to identify women at high risk of developing breast or ovarian cancer, while OncoType DX may be used to predict recurrence in “lymph node–negative, ER-positive patients receiving adjuvant tamoxifen.” ADLM does recommend that microarray-based gene signatures should be routinely used for predicting patient outcome (C. M. Sturgeon et al., 2008).

For ovarian cancer, CA-125 screening is not recommended for asymptomatic women but is recommended (with transvaginal ultrasound) for early detection of ovarian cancer in women with hereditary syndromes. CA-125 is also recommended for distinguishing benign from malignant masses and may be used to monitor chemotherapeutic response. Measurement of CA-125 during follow-up visits is recommended if the patient’s initial values were increased. CA-125 measurement is also recommended during primary therapy. Other biomarkers such as inhibin and hCG cannot be recommended at this time (C. M. Sturgeon et al., 2008).

North American Neuroendocrine Tumor Society (NANETS)

The North American Neuroendocrine Tumor Society notes that although most of its expert panel’s members measure CgA and/or pancreastatin, a majority of them believed that “these

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tumor markers assist in patient management only occasionally or rarely.” No consensus was reached on whether these tumor markers should be routinely measured (NANETS, 2017).

In 2020, NANETS published a guideline focusing on the “Surveillance and Medical Management of Pancreatic Neuroendocrine Tumors.” In it, they authors remark that “Use of nonspecific tumor markers such as CgA, pancreastatin (PcSt), and others is not recommended for routine use in patients with pNETs,” stating that these marker analyses “rarely, if ever” influence treatment (Halfdanarson et al., 2020).

American Association for the Study of Liver Diseases (AASLD)

The American Association for the Study of Liver Diseases provided updated guidance on the prevention, diagnosis, and treatment of hepatocellular carcinoma in May, 2023. This guideline states that several promising biomarkers are being investigated for potential utility in HCC surveillance, but most have not been sufficiently validated for this purpose, with the exception of AFP-L3% and DCP. Hence, “AASLD does not recommend routine use of CT- or MRI-based imaging and tumor biomarkers, outside of AFP, for HCC surveillance in at-risk patients with cirrhosis or chronic HBV (Level 5, Weak Recommendation).” While AFP may be used for screening purposes, AASLD does not yet support its diagnostic use, stating that “the diagnosis of HCC should be based on noninvasive imaging criteria or pathology. Biomarkers, such as AFP, are not sufficiently accurate to make a diagnosis of HCC” (Singal et al., 2023). Finally, AASLD advises use of the BCLC (Barcelona Liver Clinic Cancer) system for disease staging, which incorporates AFP levels.

The association also published updated guidance on primary sclerosing cholangitis and cholangiocarcinoma in February, 2023. This guideline acknowledges that CA 19-9 is the most common serum marker associated with cholangiocarcinoma (CCA), but is limited by variable sensitivity and specificity, particularly because it may be elevated in many benign and other malignant conditions (Bowlus et al., 2023).

American Thyroid Association (ATA)

The American Thyroid Association cannot recommend for or against routine measurement of serum calcitonin in patients with thyroid nodules. Furthermore, ATA cautions that unusual levels of calcitonin may occur with a variety of other conditions apart from medullary thyroid carcinoma, and notes that calcitonin levels are often elevated in young children and males compared to females (Haugen et al., 2016; Wells et al., 2015).

International Mesothelioma Interest Group

The Interest Group considers the following biomarkers to be “very useful”: Calretinin, Cytokeratin 5/6, WT1, Podoplanin (D2-40) (for epitheloid mesothelioma), Claudin four, MOC31, B72.3, CEA, BER-EP4, BG8 (Lewis^Y), TTF-1, and Napsin A (for lung adenocarcinoma) (Husain et al., 2018).

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VI. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: <https://www.cms.gov/medicare-coverage-database/search.aspx>. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

There are numerous FDA-approved tests for the assessment of serum tumor markers. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81479	Unlisted molecular pathology procedure
81538	Oncology (lung), mass spectrometric 8-protein signature, including amyloid A, utilizing serum, prognostic and predictive algorithm reported as good versus poor overall survival Proprietary test: VeriStrat® Lab/manufacturer: Biodesix, Inc
81599	Unlisted multianalyte assay with algorithmic analysis
82105	Alpha-fetoprotein (AFP); serum
82107	Alpha-fetoprotein (AFP); AFP-L3 fraction isoform and total AFP (including ratio)
82232	Beta-2 microglobulin
82308	Calcitonin
82378	Carcinoembryonic antigen (CEA)
83520	Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified
83521	Immunoglobulin light chains (i.e., kappa, lambda), free, each
83615	Lactate dehydrogenase (LD), (LDH);

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CPT	Code Description
83789	Mass spectrometry and tandem mass spectrometry (e.g., MS, MS/MS, MALDI, MS-TOF, QTOF), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen
83880	Natriuretic peptide
83950	Oncoprotein; HER-2/neu
83951	Oncoprotein; des-gamma-carboxy-prothrombin (DCP)
84075	Phosphatase, alkaline
84078	Phosphatase, alkaline; heat stable (total not included)
84080	Phosphatase, alkaline; isoenzymes
84484	Troponin, quantitative
84702	Gonadotropin, chorionic (hCG); quantitative
84703	Gonadotropin, chorionic (hCG); qualitative
84704	Gonadotropin, chorionic (hCG); free beta chain
84999	Unlisted chemistry procedure
86300	Immunoassay for tumor antigen, quantitative; CA 15-3 (27.29)
86301	Immunoassay for tumor antigen, quantitative; CA 19-9
86304	Immunoassay for tumor antigen, quantitative; CA 125
86305	Human epididymis protein 4 (HE4)
86316	Immunoassay for tumor antigen, other antigen, quantitative (e.g., CA 50, 72-4, 549), each
86336	Inhibin A
0404U	Oncology (breast), semiquantitative measurement of thymidine kinase activity by immunoassay, serum, results reported as risk of disease progression Proprietary test: Divitum®Tka Lab/Manufacturer: Biovica Inc

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Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

- AAAAI. *Systemic Mastocytosis*. <https://www.aaaai.org/conditions-treatments/related-conditions/systemic-mastocytosis>
- ACS. (2018a). *What Is Multiple Myeloma?* <https://www.cancer.org/cancer/multiple-myeloma/about/what-is-multiple-myeloma.html>
- ACS. (2018b). *What Is Waldenstrom Macroglobulinemia?* <https://www.cancer.org/cancer/waldenstrom-macroglobulinemia/about/what-is-wm.html>
- Akar, H., Seldin, D. C., Magnani, B., O'Hara, C., Berk, J. L., Schoonmaker, C., Cabral, H., Dember, L. M., Sanchorawala, V., Connors, L. H., Falk, R. H., & Skinner, M. (2005).

Reimbursement Policy

- Quantitative serum free light chain assay in the diagnostic evaluation of AL amyloidosis. *Amyloid*, 12(4), 210-215. <https://doi.org/10.1080/13506120500352339>
- ASPIRA. *OVA1 Products*. <https://aspirawh.com/ova-products/>
- ASPIRA. *OvaWatch*. <https://aspirawh.com/ovawatch/>
- Berrebi, A., Shvidel, L., Arditti, F. D., Bassous, L., Haran, M., & Shtalrid, M. (2009). The Significance of Elevated Beta 2-Microglobulin (b2-m) in B-CLL: Evidence of in Vitro b2-m Secretion Following Activation of B-CLL Cells. *Blood*, 114(22), 4380. <http://www.bloodjournal.org/content/114/22/4380.abstract>
- BeScreened. (2023). BeScreened. <https://bescreened.com/>
- Bhole, M. V., Sadler, R., & Ramasamy, K. (2014). Serum-free light-chain assay: clinical utility and limitations. *Ann Clin Biochem*, 51(Pt 5), 528-542. <https://doi.org/10.1177/0004563213518758>
- Bind, M. K., Mishra, R. R., Kumar, V., Misra, V., & Singh, P. A. (2021). Serum CA 19-9 and CA 125 as a diagnostic marker in carcinoma of gallbladder. *Indian J Pathol Microbiol*, 64(1), 65-68. <https://pubmed.ncbi.nlm.nih.gov/33433411/>
- Bowlus, C. L., Arrive, L., Bergquist, A., Deneau, M., Forman, L., Ilyas, S. I., Lunsford, K. E., Martinez, M., Sapisochin, G., Shroff, R., Tabibian, J. H., & Assis, D. N. (2023). AASLD practice guidance on primary sclerosing cholangitis and cholangiocarcinoma. *Hepatology*, 77(2), 659-702. <https://doi.org/10.1002/hep.32771>
- Cautha, S., Gupta, S., Hanif, A., Moirangthem, V., & Jain, K. (2022). Lymphoplasmacytic Lymphoma with Only Lambda Light Chain Monoclonal Paraprotein Expression. *Eur J Case Rep Intern Med*, 9(2), 003106. https://doi.org/10.12890/2022_003106
- Caviglia, G. P., Abate, M. L., Petrini, E., Gaia, S., Rizzetto, M., & Smedile, A. (2016). Highly sensitive alpha-fetoprotein, Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein and des-gamma-carboxyprothrombin for hepatocellular carcinoma detection. *Hepatol Res*, 46(3), E130-135. <https://doi.org/10.1111/hepr.12544>
- Chappuis, P. O., Dieterich, B., Sciretta, V., Lohse, C., Bonnefoi, H., Remadi, S., & Sappino, A. P. (2001). Functional evaluation of plasmin formation in primary breast cancer. *J Clin Oncol*, 19(10), 2731-2738. <https://doi.org/10.1200/jco.2001.19.10.2731>
- Chaulin, A. M. (2022). Biology of Cardiac Troponins: Emphasis on Metabolism. *Biology (Basel)*, 11(3). <https://doi.org/10.3390/biology11030429>
- Chen, F., Shen, J., Wang, J., Cai, P., & Huang, Y. (2018). Clinical analysis of four serum tumor markers in 458 patients with ovarian tumors: diagnostic value of the combined use of HE4, CA125, CA19-9, and CEA in ovarian tumors. *Cancer Manag Res*, 10, 1313-1318. <https://doi.org/10.2147/cmar.S155693>
- Chen, Y., Xie, Y., Xu, L., Zhan, S., Xiao, Y., Gao, Y., Wu, B., & Ge, W. (2017). Protein content and functional characteristics of serum-purified exosomes from patients with colorectal cancer revealed by quantitative proteomics. *Int J Cancer*, 140(4), 900-913. <https://doi.org/10.1002/ijc.30496>
- Cheng, J., Wang, W., Zhang, Y., Liu, X., Li, M., Wu, Z., Liu, Z., Lv, Y., & Wang, B. (2014). Prognostic role of pre-treatment serum AFP-L3% in hepatocellular carcinoma: systematic

Reimbursement Policy

- review and meta-analysis. *PLoS One*, 9(1), e87011.
<https://doi.org/10.1371/journal.pone.0087011>
- Di Castelnuovo, A., Veronesi, G., Costanzo, S., Zeller, T., Schnabel, R. B., de Curtis, A., Salomaa, V., Borchini, R., Ferrario, M., Giampaoli, S., Kee, F., Soderberg, S., Niiranen, T., Kuulasmaa, K., de Gaetano, G., Donati, M. B., Blankenberg, S., Iacoviello, L., & BiomarCa, R. E. I. (2019). NT-proBNP (N-Terminal Pro-B-Type Natriuretic Peptide) and the Risk of Stroke. *Stroke*, 50(3), 610-617. <https://doi.org/10.1161/STROKEAHA.118.023218>
- Dispenzieri, A. (2022). *Clinical presentation, laboratory manifestations, and diagnosis of immunoglobulin light chain (AL) amyloidosis*. <https://www.uptodate.com/contents/clinical-presentation-laboratory-manifestations-and-diagnosis-of-immunoglobulin-light-chain-al-amyloidosis>
- Dorigo, O., & Berek, J. S. (2011). Personalizing CA125 levels for ovarian cancer screening. *Cancer Prev Res (Phila)*, 4(9), 1356-1359. <https://doi.org/10.1158/1940-6207.Capr-11-0378>
- Duffy, M. J. (2001). Carcinoembryonic Antigen as a Marker for Colorectal Cancer: Is It Clinically Useful? *Clinical Chemistry*, 47(4), 624. <https://doi.org/10.1093/clinchem/47.4.624>
- Farkkila, A., Koskela, S., Bryk, S., Alfthan, H., Butzow, R., Leminen, A., Puistola, U., Tapanainen, J. S., Heikinheimo, M., Anttonen, M., & Unkila-Kallio, L. (2015). The clinical utility of serum anti-Mullerian hormone in the follow-up of ovarian adult-type granulosa cell tumors--A comparative study with inhibin B. *Int J Cancer*, 137(7), 1661-1671.
<https://doi.org/10.1002/ijc.29532>
- Febbo, P. G., Ladanyi, M., Aldape, K. D., De Marzo, A. M., Hammond, M. E., Hayes, D. F., Iafrate, A. J., Kelley, R. K., Marcucci, G., Ogino, S., Pao, W., Sgroi, D. C., & Birkeland, M. L. (2011). NCCN Task Force report: Evaluating the clinical utility of tumor markers in oncology. *J Natl Compr Canc Netw*, 9 Suppl 5, S1-32; quiz S33.
<https://doi.org/10.6004/jnccn.2011.0137>
- Feng, F., Tian, Y., Xu, G., Liu, Z., Liu, S., Zheng, G., Guo, M., Lian, X., Fan, D., & Zhang, H. (2017). Diagnostic and prognostic value of CEA, CA19-9, AFP and CA125 for early gastric cancer. *BMC Cancer*, 17(1), 737. <https://doi.org/10.1186/s12885-017-3738-y>
- Foekens, J. A., Peters, H. A., Look, M. P., Portengen, H., Schmitt, M., Kramer, M. D., Brunner, N., Janicke, F., Meijer-van Gelder, M. E., Henzen-Logmans, S. C., van Putten, W. L., & Klijn, J. G. (2000). The urokinase system of plasminogen activation and prognosis in 2780 breast cancer patients. *Cancer Res*, 60(3), 636-643.
<https://pubmed.ncbi.nlm.nih.gov/10676647/>
- Foukakis, T., & Bergh, J. (2022). Prognostic and predictive factors in early, nonmetastatic breast cancer - UpToDate. In D. Hayes (Ed.), *UpToDate*.
<https://www.uptodate.com/contents/prognostic-and-predictive-factors-in-early-non-metastatic-breast-cancer>
- Gershenson, D. (2022). *Sex cord-stromal tumors of the ovary: Epidemiology, clinical features, and diagnosis in adults*. <https://www.uptodate.com/contents/sex-cord-stromal-tumors-of-the-ovary-epidemiology-clinical-features-and-diagnosis-in-adults>
- Gilligan, T. D., Seidenfeld, J., Basch, E. M., Einhorn, L. H., Fancher, T., Smith, D. C., Stephenson, A. J., Vaughn, D. J., Cosby, R., & Hayes, D. F. (2010). American Society of

Reimbursement Policy

- Clinical Oncology Clinical Practice Guideline on uses of serum tumor markers in adult males with germ cell tumors. *J Clin Oncol*, 28(20), 3388-3404.
<https://doi.org/10.1200/jco.2009.26.4481>
- Halfdanarson, T. R., Strosberg, J. R., Tang, L., Bellizzi, A. M., Bergsland, E. K., O'Dorisio, T. M., Halperin, D. M., Fishbein, L., Eads, J., Hope, T. A., Singh, S., Salem, R., Metz, D. C., Naraev, B. G., Reidy-Lagunes, D. L., Howe, J. R., Pommier, R. F., Menda, Y., & Chan, J. A. (2020). The North American Neuroendocrine Tumor Society Consensus Guidelines for Surveillance and Medical Management of Pancreatic Neuroendocrine Tumors. *Pancreas*, 49(7), 863-881. <https://doi.org/10.1097/mpa.0000000000001597>
- Harris, L. N., Ismaila, N., McShane, L. M., Andre, F., Collyar, D. E., Gonzalez-Angulo, A. M., Hammond, E. H., Kuderer, N. M., Liu, M. C., Mennel, R. G., Van Poznak, C., Bast, R. C., & Hayes, D. F. (2016). Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol*, 34(10), 1134-1150.
<https://doi.org/10.1200/jco.2015.65.2289>
- Harvey, R. A. (2023). *Human chorionic gonadotropin: Biochemistry and measurement in pregnancy and disease*. <https://www.uptodate.com/contents/human-chorionic-gonadotropin-testing-in-pregnancy-and-gestational-trophoblastic-disease-and-causes-of-low-persistent-levels>
- Haugen, B. R., Alexander, E. K., Bible, K. C., Doherty, G. M., Mandel, S. J., Nikiforov, Y. E., Pacini, F., Randolph, G. W., Sawka, A. M., Schlumberger, M., Schuff, K. G., Sherman, S. I., Sosa, J. A., Steward, D. L., Tuttle, R. M., & Wartofsky, L. (2016). 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*, 26(1), 1-133.
<https://doi.org/10.1089/thy.2015.0020>
- Hotakainen, K., Ljungberg, B., Paju, A., Rasmuson, T., Alfthan, H., & Stenman, U. H. (2002). The free beta-subunit of human chorionic gonadotropin as a prognostic factor in renal cell carcinoma. *Br J Cancer*, 86(2), 185-189. <https://doi.org/10.1038/sj.bjc.6600050>
- Hottinger, A., & Hormigo, A. (2011). Serum Biomarkers. In *Encyclopedia of Cancer* (pp. 3390-3394). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-16483-5_5269
- Husain, A. N., Colby, T. V., Ordonez, N. G., Allen, T. C., Attanoos, R. L., Beasley, M. B., Butnor, K. J., Chirieac, L. R., Churg, A. M., Dacic, S., Galateau-Salle, F., Gibbs, A., Gown, A. M., Krausz, T., Litzky, L. A., Marchevsky, A., Nicholson, A. G., Roggli, V. L., Sharma, A. K., . . . Wick, M. R. (2018). Guidelines for Pathologic Diagnosis of Malignant Mesothelioma 2017 Update of the Consensus Statement From the International Mesothelioma Interest Group. *Arch Pathol Lab Med*, 142(1), 89-108.
<https://doi.org/10.5858/arpa.2017-0124-ra>
- Isaksson, S., Jönsson, P., Monsef, N., Brunnström, H., Bendahl, P. O., Jönsson, M., Staaf, J., & Planck, M. (2017). CA 19-9 and CA 125 as potential predictors of disease recurrence in resectable lung adenocarcinoma. *PLoS One*, 12(10), e0186284.
<https://doi.org/10.1371/journal.pone.0186284>

Reimbursement Policy

- Katou, H., Kanno, T., Hoshino, M., Hagihara, Y., Tanaka, H., Kawai, T., Hasegawa, K., Naiki, H., & Goto, Y. (2002). The role of disulfide bond in the amyloidogenic state of beta(2)-microglobulin studied by heteronuclear NMR. *Protein Sci*, 11(9), 2218-2229. <https://doi.org/10.1110/ps.0213202>
- Katzmann, J. A., Clark, R. J., Abraham, R. S., Bryant, S., Lymp, J. F., Bradwell, A. R., & Kyle, R. A. (2002). Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. *Clin Chem*, 48(9), 1437-1444. <https://www.ncbi.nlm.nih.gov/pubmed/12194920>
- Kim, N. H., Lee, M. Y., Park, J. H., Park, D. I., Sohn, C. I., Choi, K., & Jung, Y. S. (2017). Serum CEA and CA 19-9 Levels are Associated with the Presence and Severity of Colorectal Neoplasia. *Yonsei Med J*, 58(5), 918-924. <https://doi.org/10.3349/ymj.2017.58.5.918>
- Kindler, H. L., Ismaila, N., Armato, S. G., Bueno, R., Hesdorffer, M., Jahan, T., Jones, C. M., Miettinen, M., Pass, H., Rimner, A., Rusch, V., Serman, D., Thomas, A., & Hassan, R. (2018). Treatment of Malignant Pleural Mesothelioma: American Society of Clinical Oncology Clinical Practice Guideline. *Journal of Clinical Oncology*, 36(13), 1343-1373. <https://doi.org/10.1200/JCO.2017.76.6394>
- Kumar, S., Dispenzieri, A., Katzmann, J. A., Larson, D. R., Colby, C. L., Lacy, M. Q., Hayman, S. R., Buadi, F. K., Leung, N., Zeldenrust, S. R., Ramirez-Alvarado, M., Clark, R. J., Kyle, R. A., Rajkumar, S. V., & Gertz, M. A. (2010). Serum immunoglobulin free light-chain measurement in primary amyloidosis: prognostic value and correlations with clinical features. *Blood*, 116(24), 5126-5129. <https://doi.org/10.1182/blood-2010-06-290668>
- Kyrtsonis MC, K. E., Bartzis V, Pessah I, Nikolaou E, Karalis V, Maltezas D, Panayiotidis P, Harding S. (2012). Monoclonal Immunoglobulin. In *Multiple Myeloma - A Quick Reflection on the Fast Progress*. <https://doi.org/10.5772/55855>
- Leru, P. M. (2022). Evaluation and Classification of Mast Cell Disorders: A Difficult to Manage Pathology in Clinical Practice. *Cureus*, 14(2), e22177. <https://doi.org/10.7759/cureus.22177>
- Li, A. J. (2023). *Serum biomarkers for evaluation of an adnexal mass for epithelial carcinoma of the ovary, fallopian tube, or peritoneum*. <https://www.uptodate.com/contents/serum-biomarkers-for-evaluation-of-an-adnexal-mass-for-epithelial-carcinoma-of-the-ovary-fallopian-tube-or-peritoneum>
- Li, J., Yin, M., Song, W., Cui, F., Wang, W., Wang, S., & Zhu, H. (2018). B Subunit of Human Chorionic Gonadotropin Promotes Tumor Invasion and Predicts Poor Prognosis of Early-Stage Colorectal Cancer. *Cell Physiol Biochem*, 45(1), 237-249. <https://doi.org/10.1159/000486770>
- Liu, R., Cao, J., Gao, X., Zhang, J., Wang, L., Wang, B., Guo, L., Hu, X., & Wang, Z. (2016). Overall survival of cancer patients with serum lactate dehydrogenase greater than 1000 IU/L. *Tumour Biol*, 37(10), 14083-14088. <https://doi.org/10.1007/s13277-016-5228-2>
- Lucarelli, G., Ditunno, P., Bettocchi, C., Vavallo, A., Rutigliano, M., Galleggiante, V., Larocca, A. M., Castellano, G., Gesualdo, L., Grandaliano, G., Selvaggi, F. P., & Battaglia, M. (2014). Diagnostic and prognostic role of preoperative circulating CA 15-3, CA 125, and beta-2 microglobulin in renal cell carcinoma. *Dis Markers*, 2014, 689795. <https://doi.org/10.1155/2014/689795>

Reimbursement Policy

- MagArray. (2023). REVEAL. <https://magarray.com/reveal/#>
- Magnani, J. L. (2004). The discovery, biology, and drug development of sialyl Lea and sialyl Lex. *Archives of Biochemistry and Biophysics*, 426(2), 122-131. <https://doi.org/10.1016/j.abb.2004.04.008>
- Malmstrom, P., Bendahl, P. O., Boiesen, P., Brunner, N., Idvall, I., & Ferno, M. (2001). S-phase fraction and urokinase plasminogen activator are better markers for distant recurrences than Nottingham Prognostic Index and histologic grade in a prospective study of premenopausal lymph node-negative breast cancer. *J Clin Oncol*, 19(7), 2010-2019. <https://doi.org/10.1200/jco.2001.19.7.2010>
- Marcillac, I., Troalen, F., Bidart, J.-M., Ghillani, P., Ribrag, V., Escudier, B., Malassagne, B., Droz, J.-P., Lhommé, C., Rougier, P., Duvillard, P., Prade, M., Lugagne, P.-M., Richard, F., Poynard, T., Bohuon, C., Wands, J., & Bellet, D. (1992). Free Human Chorionic Gonadotropin β Subunit in Gonadal and Nongonadal Neoplasms. *Cancer Research*, 52(14), 3901. <http://cancerres.aacrjournals.org/content/52/14/3901.abstract>
- Marcinko, T. M., Dong, J., LeBlanc, R., Daborowski, K. V., & Vachet, R. W. (2017). Small molecule-mediated inhibition of β -2-microglobulin-based amyloid fibril formation. *J Biol Chem*, 292(25), 10630-10638. <https://doi.org/10.1074/jbc.M116.774083>
- Merlini, G., Wechalekar, A. D., & Palladini, G. (2013). Systemic light chain amyloidosis: an update for treating physicians. *Blood*, 121(26), 5124-5130. <https://doi.org/10.1182/blood-2013-01-453001>
- Moreau AS, L. X., Manning R, Coiteux V, Darre S, Hatjiharisi E, Hunter Z, Jia X, Ngo H, O'Sullivan G, Santos D, Treon S, Facon T, Anderson K, Ghobrial I. (2006). Serum Free Light Chain in Waldenstrom Macroglobulinemia. <https://doi.org/10.1182/blood.V108.11.2420.2420>
- NANETS. (2017). The North American Neuroendocrine Tumor Society Consensus Guidelines for Surveillance and Medical Management of Midgut Neuroendocrine Tumors. <https://doi.org/10.1097%2FMPA.0000000000000850>
- NCCN. (2023a). *Biomarkers Compendium*. <https://www.nccn.org/compendia-templates/compendia/biomarkers-compendium>
- NCCN. (2023b). *NCCN Clinical Practice Guidelines in Oncology*. https://www.nccn.org/professionals/physician_gls/default.aspx
- NCCN. (2023c). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Multiple Myeloma Version 2.2024*. https://www.nccn.org/professionals/physician_gls/pdf/myeloma.pdf#Page=9
- NCCN. (2023d). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Systemic Light Chain Amyloidosis Version 1.2024*. https://www.nccn.org/professionals/physician_gls/pdf/amyloidosis.pdf
- NCI. (2021). *Tumor Markers*. <https://www.cancer.gov/about-cancer/diagnosis-staging/diagnosis/tumor-markers-fact-sheet>
- Oyaert, M., Boone, E., De Ceuninck, L., Moreau, E., Van Dorpe, J., Vanpoucke, H., & Deeren, D. (2014). Clonal multicentric Castleman's disease with increased free Kappa light chains in

Reimbursement Policy

- a patient with systemic lupus erythematosus. *Ann Hematol*, 93(7), 1255-1257.
<https://doi.org/10.1007/s00277-013-1962-3>
- Park, S. J., Jang, J. Y., Jeong, S. W., Cho, Y. K., Lee, S. H., Kim, S. G., Cha, S. W., Kim, Y. S., Cho, Y. D., Kim, H. S., Kim, B. S., Park, S., & Bang, H. I. (2017). Usefulness of AFP, AFP-L3, and PIVKA-II, and their combinations in diagnosing hepatocellular carcinoma. *Medicine (Baltimore)*, 96(11), e5811. <https://doi.org/10.1097/md.0000000000005811>
- Payne, V., & Kam, P. C. (2004). Mast cell tryptase: a review of its physiology and clinical significance. *Anaesthesia*, 59(7), 695-703. <https://doi.org/10.1111/j.1365-2044.2004.03757.x>
- Pejler, G., Ronnberg, E., Waern, I., & Wernersson, S. (2010). Mast cell proteases: multifaceted regulators of inflammatory disease. *Blood*, 115(24), 4981-4990.
<https://doi.org/10.1182/blood-2010-01-257287>
- Perfetto, F., Bergesio, F., Emdin, M., & Cappelli, F. (2014). Troponins in cardiac amyloidosis: multipurpose markers. *Nat Rev Cardiol*, 11(3), 179.
<https://doi.org/10.1038/nrcardio.2013.129-c1>
- Pinzani, P., D'Argenio, V., Del Re, M., Pellegrini, C., Cucchiara, F., Salvianti, F., & Galbiati, S. (2021). Updates on liquid biopsy: current trends and future perspectives for clinical application in solid tumors. *Clin Chem Lab Med*, 59(7), 1181-1200.
<https://doi.org/10.1515/cclm-2020-1685>
- Pucino, V., Bombardieri, M., Pitzalis, C., & Mauro, C. (2017). Lactate at the crossroads of metabolism, inflammation, and autoimmunity. *European Journal of Immunology*, 47(1), 14-21. <https://doi.org/10.1002/eji.201646477>
- Qin, J., Yang, Q., Ye, H., Wang, K., Zhang, M., Zhu, J., Wang, X., Dai, L., Wang, P., & Zhang, J. (2020). Using Serological Proteome Analysis to Identify and Evaluate Anti-GRP78 Autoantibody as Biomarker in the Detection of Gastric Cancer. *J Oncol*, 2020, 9430737.
<https://doi.org/10.1155/2020/9430737>
- Raby, B. (2023). *Personalized medicine*. <https://www.uptodate.com/contents/personalized-medicine>
- Ryu, T., Takami, Y., Wada, Y., Tateishi, M., Matsushima, H., Mikagi, K., & Saitsu, H. (2017). Double- and Triple-Positive Tumor Markers Predict Early Recurrence and Poor Survival in Patients with Hepatocellular Carcinoma within the Milan Criteria and Child-Pugh Class A. *J Gastrointest Surg*, 21(6), 957-966. <https://doi.org/10.1007/s11605-017-3394-1>
- Santos Schraiber, L. d., de Mattos, A. A., Zanotelli, M. L., Cantisani, G. P., Brandao, A. B., Marroni, C. A., Kiss, G., Ernani, L., & Santos Marcon, P. d. (2016). Alpha-fetoprotein Level Predicts Recurrence After Transplantation in Hepatocellular Carcinoma. *Medicine (Baltimore)*, 95(3), e2478. <https://doi.org/10.1097/md.0000000000002478>
- Schefer, H., Mattmann, S., & Joss, R. A. (1998). Hereditary persistence of α -fetoprotein Case report and review of the literature. *Annals of Oncology*, 9(6), 667-672.
<https://doi.org/10.1023/A:1008243311122>
- Seo, S., Hong, J. Y., Yoon, S., Yoo, C., Park, J. H., Lee, J. B., Park, C. S., Huh, J., Lee, Y., Kim, K. W., Ryu, J. S., Kim, S. J., Kim, W. S., Yoon, D. H., & Suh, C. (2016). Prognostic significance of serum beta-2 microglobulin in patients with diffuse large B-cell lymphoma in

Reimbursement Policy

- the rituximab era. *Oncotarget*, 7(47), 76934-76943.
<https://doi.org/10.18632/oncotarget.12734>
- Sharma, S., Jackson, P. G., & Makan, J. (2004). Cardiac troponins. *J Clin Pathol*, 57(10), 1025-1026. <https://doi.org/10.1136/jcp.2003.015420>
- Sharma, U., Pal, D., & Prasad, R. (2014). Alkaline phosphatase: an overview. *Indian J Clin Biochem*, 29(3), 269-278. <https://doi.org/10.1007/s12291-013-0408-y>
- Singal, A. G., Llovet, J. M., Yarchoan, M., Mehta, N., Heimbach, J. K., Dawson, L. A., Jou, J. H., Kulik, L. M., Agopian, V. G., Marrero, J. A., Mendiratta-Lala, M., Brown, D. B., Rilling, W. S., Goyal, L., Wei, A. C., & Taddei, T. H. (2023). AASLD Practice Guidance on prevention, diagnosis, and treatment of hepatocellular carcinoma. *Hepatology*.
<https://doi.org/10.1097/HEP.0000000000000466>
- Stankowski-Drengler, T., Gertz, M. A., Katzmann, J. A., Lacy, M. Q., Kumar, S., Leung, N., Hayman, S. R., Buadi, F., Kyle, R. A., Rajkumar, S. V., & Dispenzieri, A. (2010). Serum immunoglobulin free light chain measurements and heavy chain isotype usage provide insight into disease biology in patients with POEMS syndrome. *Am J Hematol*, 85(6), 431-434. <https://doi.org/10.1002/ajh.21707>
- Stephens, R. W., Brunner, N., Janicke, F., & Schmitt, M. (1998). The urokinase plasminogen activator system as a target for prognostic studies in breast cancer. *Breast Cancer Res Treat*, 52(1-3), 99-111. https://doi.org/10.1007/978-1-4615-5195-9_15
- Stoffel, E. M., McKernin, S. E., Brand, R., Canto, M., Goggins, M., Moravek, C., Nagarajan, A., Petersen, G. M., Simeone, D. M., Yurgelun, M., & Khorana, A. A. (2018). Evaluating Susceptibility to Pancreatic Cancer: ASCO Provisional Clinical Opinion. *Journal of Clinical Oncology*, 37(2), 153-164. <https://doi.org/10.1200/JCO.18.01489>
- Strosberg, J. (2022). *Diagnosis of carcinoid syndrome and tumor localization*.
<https://www.uptodate.com/contents/diagnosis-of-the-carcinoid-syndrome-and-tumor-localization>
- Sturgeon, C. M., Duffy, M. J., Hofmann, B. R., Lamerz, R., Fritsche, H. A., Gaarenstroom, K., Bonfrer, J., Ecke, T. H., Grossman, H. B., Hayes, P., Hoffmann, R. T., Lerner, S. P., Lohe, F., Louhimo, J., Sawczuk, I., Taketa, K., & Diamandis, E. P. (2010). National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for use of tumor markers in liver, bladder, cervical, and gastric cancers. *Clin Chem*, 56(6), e1-48.
<https://doi.org/10.1373/clinchem.2009.133124>
- Sturgeon, C. M., Duffy, M. J., Stenman, U. H., Lilja, H., Brunner, N., Chan, D. W., Babaian, R., Bast, R. C., Jr., Dowell, B., Esteva, F. J., Haglund, C., Harbeck, N., Hayes, D. F., Holten-Andersen, M., Klee, G. G., Lamerz, R., Looijenga, L. H., Molina, R., Nielsen, H. J., . . . Diamandis, E. P. (2008). National Academy of Clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers. *Clin Chem*, 54(12), e11-79. <https://doi.org/10.1373/clinchem.2008.105601>
- Sturgeon, C. M., Hoffman, B. R., Chan, D. W., Ch, ng, S.-L., Hammond, E., Hayes, D. F., Liotta, L. A., Petricoin, E. F., Schmitt, M., Semmes, O. J., Söletormos, G., van der Merwe, E., & Diamandis, E. P. (2008). National Academy of Clinical Biochemistry Laboratory

Reimbursement Policy

- Medicine Practice Guidelines for Use of Tumor Markers in Clinical Practice: Quality Requirements. *Clinical Chemistry*, 54(8), e1. <https://doi.org/10.1373/clinchem.2007.094144>
- Szulc, P., Bauer, D. C., Dempster, D. W., Luckey, M., & Cauley, J. A. (2013). Osteoporosis. *I*. <https://doi.org/https://doi.org/10.1016/B978-0-12-415853-5.00067-4>
- Thio, Q., Karhade, A. V., Notman, E., Raskin, K. A., Lozano-Calderon, S. A., Ferrone, M. L., Bramer, J. A. M., & Schwab, J. H. (2020). Serum alkaline phosphatase is a prognostic marker in bone metastatic disease of the extremity. *J Orthop*, 22, 346-351. <https://doi.org/10.1016/j.jor.2020.08.008>
- Tian, T., Gao, J., Li, N., Li, Y., Lu, M., Li, Z., Lu, Z., Li, J., & Shen, L. (2016). Circulating Chromogranin A as A Marker for Monitoring Clinical Response in Advanced Gastroenteropancreatic Neuroendocrine Tumors. *PLoS One*, 11(5), e0154679. <https://doi.org/10.1371/journal.pone.0154679>
- Tormey, W. P., Byrne, B., Hill, A. D., Sherlock, M., & Thompson, C. J. (2017). Should serum calcitonin be routinely measured in patients presenting with thyroid nodule? *Minerva Endocrinol*, 42(4), 306-310. <https://doi.org/10.23736/s0391-1977.17.02566-4>
- Tosi, P., Tomassetti, S., Merli, A., & Polli, V. (2013). Serum free light-chain assay for the detection and monitoring of multiple myeloma and related conditions. *Ther Adv Hematol*, 4(1), 37-41. <https://doi.org/10.1177/2040620712466863>
- Tuttle, R. M. (2022). *Medullary thyroid cancer: Clinical manifestations, diagnosis, and staging*. <https://www.uptodate.com/contents/medullary-thyroid-cancer-clinical-manifestations-diagnosis-and-staging>
- Van Poznak, C., Somerfield, M. R., Bast, R. C., Cristofanilli, M., Goetz, M. P., Gonzalez-Angulo, A. M., Hicks, D. G., Hill, E. G., Liu, M. C., Lucas, W., Mayer, I. A., Mennel, R. G., Symmans, W. F., Hayes, D. F., & Harris, L. N. (2015). Use of Biomarkers to Guide Decisions on Systemic Therapy for Women With Metastatic Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol*, 33(24), 2695-2704. <https://doi.org/10.1200/jco.2015.61.1459>
- Venner, C. P. (2019). AL amyloidosis cardiac staging updated using BNP. *Blood*, 133(3), 184-185. <https://doi.org/10.1182/blood-2018-10-882159>
- Walentowicz, P., Krintus, M., Sadlecki, P., Grabiec, M., Mankowska-Cyl, A., Sokup, A., & Walentowicz-Sadlecka, M. (2014). Serum inhibin A and inhibin B levels in epithelial ovarian cancer patients. *PLoS One*, 9(3), e90575. <https://doi.org/10.1371/journal.pone.0090575>
- Weber, M., & Hamm, C. (2006). Role of B-type natriuretic peptide (BNP) and NT-proBNP in clinical routine. *Heart*, 92(6), 843-849. <https://doi.org/10.1136/hrt.2005.071233>
- Wells, S. A., Jr., Asa, S. L., Dralle, H., Elisei, R., Evans, D. B., Gagel, R. F., Lee, N., Machens, A., Moley, J. F., Pacini, F., Raue, F., Frank-Raue, K., Robinson, B., Rosenthal, M. S., Santoro, M., Schlumberger, M., Shah, M., & Waguespack, S. G. (2015). Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid*, 25(6), 567-610. <https://doi.org/10.1089/thy.2014.0335>
- Wu, D., Lim, M. S., & Jaffe, E. S. (2018). Pathology of Castleman Disease. *Hematol Oncol Clin North Am*, 32(1), 37-52. <https://doi.org/10.1016/j.hoc.2017.09.004>

Reimbursement Policy

Wu, M., Liu, H., Liu, Z., Liu, C., Zhang, A., & Li, N. (2018). Analysis of serum alpha-fetoprotein (AFP) and AFP-L3 levels by protein microarray. *J Int Med Res*, 46(10), 4297-4305. <https://doi.org/10.1177/0300060518789304>

Yang, X., Yang, Y., Li, Z., Cheng, C., Yang, T., Wang, C., Liu, L., & Liu, S. (2015). Diagnostic value of circulating chromogranin a for neuroendocrine tumors: a systematic review and meta-analysis. *PLoS One*, 10(4), e0124884. <https://doi.org/10.1371/journal.pone.0124884>