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Blood and Other Biomarker Tests for Cancer Patients

INDEX

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1. **Hyper coagulation**: fibrinogen, D-Dimer, Anti-thrombin III, and Plasminogen Activator Inhibitor 1 (PAI-1) and uPA.

   The relationship between neoplastic disease and thromboembolic disorders has been recognised since I865, when Armand Trousseau first reported a high incidence of venous thrombosis in a series of patients with gastric carcinoma. (1) Three types of evidence exist that support an important relationship between blood coagulation and tumour homeostasis: clinical, histologic, and pharmacologic. Clinical and laboratory evidence focuses on the well known propensity of patients with certain forms of cancer to develop thromboembolic disease (TED) and/or disseminated intravascular coagulation (DIC), commonly observed following rapid tumour lysis or surgical manipulation. The post-mortem observations of platelet and fibrin thrombi in vessels draining tumours and the immunochemical demonstration of fibrin surrounding tumour cells have provided incontrovertible histologic evidence for an association between growing tumours and the end-products of blood coagulation, e.g., platelet aggregates and fibrin. (2)

   - **Thrombin time**

   This test measures how quickly your body makes fibrin, which is part of the blood clotting process. The test is used to assess certain conditions that involve abnormal blood clotting, (3,4) such as disseminated intravascular coagulation (DIC) (5).

   - **Fibrinogen**

   Normal fibrinogen activity results usually reflect normal blood clotting ability. Elevated levels can help predict a person's risk for developing a blood clot. Elevated levels also are often a marker of cancer progression, and tumor angiogenesis and metastatic spread of cancer. More specifically fibrin/fibrinogen deposition induces fibrinolytic activity, mainly by plasmin, resulting in extracellular matrix degradation providing fertile ground for tumor cell invasion and metastasis as well as having a direct mitogenic effect. (6-9).

   Circulating fibrinogen is a prognostic and predictive biomarker in malignant pleural mesothelioma (10). Most importantly, fibrinogen predicted treatment benefit achieved by surgery within multimodality therapy.

   Fibrinogen is also a nonspecific acute phase reactant, meaning that fibrinogen concentrations may rise sharply in any condition that causes inflammation or tissue damage.

   - **D-dimer assay**

   This test measures the amount of D-dimer in blood. This test is used to help evaluate conditions resulting from the formation of blood clots in blood vessels (11-13). This test may be used when conditions such as acute deep vein thrombosis (DVT] (11-13) or disseminated intravascular coagulation (DIC) are suspected. (14-18) Increased levels of D-dimer, which is a degradation product of cross-linked fibrin, indicate a global activation of hemostasis and fibrinolysis. In a prospective and observational cohort study, the prognostic value of D-dimer levels for overall survival and mortality risk was assessed in 1178 cancer patients. High D-dimer levels were associated with poor overall survival and increased mortality risk in cancer patients. (18) Increased D-dimer level correlated with progression (stages) and high mortality rate. Furthermore, D-dimer showed very similar or better prognostic activity than the clinically widely used classic tumor markers and suggested to use it as an additional value. (19)

   - **Urokinase plasminogen activator and Plasminogen Activator Inhibitor 1**

   For optimum management of patients with cancer, accurate assessment of prognosis is essential. The primary determinant of outcome in malignancy is the formation of distant metastases.

Urokinase plasminogen activator (uPA) is a serine protease causally involved in invasion and metastasis.
Data from model systems show that uPA is unequivocally involved in cancer dissemination. Consistent with its role in metastasis, multiple independent groups have shown that high uPA concentrations in primary breast cancers correlate with poor prognosis. Paradoxically, high concentrations of plasminogen activator inhibitor (PAI-1), an endogenous inhibitor of uPA, also correlate with poor prognosis in patients with breast cancer, including the subgroup with node-negative disease. (20) Increased levels of PAI-1 have also been reported in a number of conditions including malignancy, liver disease, the postoperative period, septic shock, the second and third trimesters of pregnancy, obesity, and coronary heart disease. (21)
An enzyme-linked immunoassays (ELISA) tested for uPA antigen and its inhibitor PAI-1 in tumor tissue extracts of 247 breast cancer patients who were enrolled in a prospective study. The relation of these data to known prognostic factors and to other variables such as DNA analysis and cathepsin D was studied. Disease-free and overall survival were analyzed according to Cox's proportional hazard model. The major new finding is that breast cancer patients with either high uPA (>2.97 ng/mg protein) or high content of the uPA inhibitor PAI-1 (>2.18 ng/mg protein) in their primary tumors have an increased risk of relapse and death. (22)
Overexpression of uPA has been proposed to be one of the mechanisms by which malignant glioma cells cause pericellular proteolysis of stromal structures during brain-tissue invasion. Growth factors, particularly epidermal growth factor and basic fibroblast growth factor (bFGF), regulate uPA synthesis in various nonglial cells. Because these factors have been associated with cancer invasion. (23)

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Tissue Factor (TF), Blood Coagulation, & Cancer

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TF over-expression has been reported in ovarian cancer [1], endometriosis [2], breast cancer [3], nonsmall cell lung carcinoma [4], prostate cancer [5], pancreatic cancer [6], melanoma [7], colorectal cancer [8], gastric cancer [9], oesophageal cancer [10], hepatocellular carcinoma [11], brain tumor glioblastoma [12], leukaemia [13], and lymphoma [14]. Accordingly, TF overexpression could be considered a biomarker for solid tumours [15].

The roles of TF in cancer have been demonstrated with several-fold relevance in relation to thrombotic condition, tumorigenesis per se and TF signalling (i.e., coagulation-dependant inflammation). Cancer linked with hypercoagulability and thrombotic risk has long been recognised by Armand Trousseau since 1865. The American Society of Haematology calling for a special session on “cancer and thrombosis” addresses its complex clinical interface of pro-thrombotic association with malignancies and prophylactic approaches. Cancer certainly could be recognized as

a pro-thrombotic risk factor, leading to, for instance, venous thromboembolism and its complication of pulmonary embolism and mortality. Namely, cancers readily induce thrombosis [16]. Enhanced TF expression typically accounts for the mode of mechanism of thrombosis accompanied by suppressed TFPI [17] and defective APC anticoagulation system. Not only tumor cellular membrane-bound TF, but also micro particle-associated TF [18] links cancer to thrombosis.

In addition, the similar hyper-coagulable state exists in cancer stem cells [19]. The critical role of TF in tumorigenesis is supported by the observations that inhibited TF expression blocks tumor growth, metastasis [20], angiogenesis [21], cell invasion [22], and many other cancer characteristics. TF per se plays important roles in cell proliferation, tumor development, and progression apart from the accompanying coagulation-dependent inflammatory environment including MMP-9 [23], growth factors (VEGF, EGF, PDGF, etc.), and adhesion molecules certainly promoting "autocrine" tumorigenesis. Either VEGF or EGF in turn stimulates sustained TF expression [24,25]. PTEN loss and tumor hypoxia readily induce TF expression [26], which could highlight TF as a major player in cancer progression. Tumor-expressed TF promotes growth by increasing cell survival and/or angiogenesis. TF and VEGF expressions mutually enhance each other [27], where VEGF is a known main angiogenic factor of cancer characteristics.

TF cytoplasmic domain has been shown to be critical for VEGF expression [28]; conversely, VEGF causes TF promoter activation and involves gene upregulation with transcription factor NFAT involvement [25]. It is of particular interest to note that the serine phosphorylated cytoplasmic domain inhibits cellular cytotoxicity [29], thereby leading to increased tumor survival and metastatic rate. In addition, increased TF cytoplasmic domain phosphorylation and PAR-2 activation significantly correlate to cancer relapse [22]. Thus, a cooperation of the phosphorylated TF cytoplasmic domain with protease signalling could account for diverse contributions of TF to metastasis and angiogenesis [30, 31]. As the proceeding of TF-initiated extrinsic pathway, the resulting FIIa generation and fibrin production are of pro-angiogenesis. Furthermore, TF/FVIIa activates BcL2 [32], and FXa inactivates caspase-3 [33], both of which inhibit apoptosis.

TF/FVIIa/FXa ternary complex possibly mediated by PAR-1/2 readily induces Erk1/2, Akt/PKB, and mTOR activation, all of which enhance the downstream signalling target phosphorylation for cancer cell undergoing anti-apoptosis [34] and cell migration [191]. FIIa-PAR signalling in metastasis [35]/angiogenesis [36] and TF/FVIIa/PARs signalling in tumor growth [37] are also evident. FIIa could be recognized as a tumor growth factor [38, 39], which is accompanied by the enhanced tumor cell cycle mediated by downregulation of p27Kip1 and upregulation of Skp2 and MiR-222 [40]. FIIa is also able to upregulate cathepsin D, which enhances angiogenesis, growth, and metastasis [41]. FIIa activates fibrinolysis inhibitors (e.g., TAFI [42] and PAI-1 [43]), further promoting cancer progression [44].

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2. Inflammation: C-reactive Protein, SED rate, IL-6 and TNF-a, Serum amyloid A

Inflammation is the body's basic response to injury. A sequence of complicated, interrelated events work to defend the body, ultimately bringing plasma proteins and phagocytes (white blood cells that engulf and consume foreign material and debris) to the injured area for the purpose of initiating tissue repair. Of the ten leading causes of mortality in the United States, chronic, low-level inflammation contributes to the pathogenesis of at least seven. These include heart disease, cancer, chronic lower respiratory disease, stroke, Alzheimer's disease, diabetes, and nephritis (1-8).

Chronic inflammation plays a multifaceted role in carcinogenesis. Mounting evidence from preclinical and clinical studies suggests that persistent inflammation functions as a driving force in the journey to cancer. The possible mechanisms by which inflammation can contribute to carcinogenesis include induction of genomic instability, alterations in epigenetic events and subsequent inappropriate gene expression, enhanced proliferation of initiated cells, resistance to apoptosis, aggressive tumor neovascularization, invasion through tumor-associated basement membrane and metastasis (7).

An inflammatory milieu consisting of infiltrated immune cells and their secretory cytokines, chemokines and growth factors contribute significantly to the invasive and metastatic traits of cancer cells (9).

Patients with malignancies often present with signs of inflammatory reactions such as elevated erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and serum amyloid-A (SAA). A significant correlation between CRP, SAA, ESR and IL-6 levels was found in patients and provided prognostic information (10)

- **Erythrocyte sedimentation rate (ESR)**

The ESR measures the rate at which the red blood cells separate from the plasma and fall to the bottom of a test tube. The rate is measured in millimetres per hour (mm/hr). If certain proteins cover red cells, these will stick to each other and cause the red cells to fall more quickly. So, a high ESR indicates inflammation, somewhere in the body.

- **TNF-α**

TNF-alpha is a growth factor for immune cells and osteoclasts, the cells that break down bone. It may be elevated in chronic infections, certain cancers and Hepatitis C. TNF-α is produced upon stimulation with cytokines such as IL-1, 1L-2, GM-CSF, TNF-α itself and with bacterial lipopolysaccharide (LPS) which is a potent inducer (10). TNF-α once produced and secreted will bind to TNF-α receptors (TNF-α R1, 55 kDa and TNF-α R2, 75 kDa), located on the plasma membrane of most cells throughout the body. Various pathological conditions are associated with the production of high levels of TNF-α. These include septic shock syndrome, cachexia (e.g. HIV, tuberculosis, cancer), autoimmune diseases, hepatitis, leukaemia, myocardial ischaemia, organ transplantation rejection, multiple sclerosis, rheumatoid arthritis, and meningococcal septicaemia. (12-15)

- **Serum amyloid A (SAA)**

SAA is a member of a group of proteins called Acute Phase Proteins which have been found in many animal species and which are thought to be part of the body's basic first line defences against infection, disease or trauma. In response to a challenge by, for example an infective organism, the body sends chemical messengers to the liver which then produces and releases SAA into the bloodstream which helps to fight off the challenge. The level of serum amyloid A, a protein previously found to behave as an acute-phase reactant, was measured by a

radioimmunoassay in 621 patients with various neoplastic diseases free of inflammatory processes. In all but eight of the 289 patients with solid tumors with distant metastases, in all patients with myelocytic leukemia with high leukocyte counts, and in all patients with advanced lymphoma, the serum amyloid A level was high. Thus the level of serum amyloid A can be used as a biochemical marker that discriminates between disseminated and localized or regional disease, and monitors the response to therapy. (16)

SAA value showed a direct correlation with the stage of cancer: it was lowest at stages 1 and 2 and highest at the metastatic stage 4. Initial SAA value had prognostic significance: a value below 10 micrograms/ml correlated with survival advantage, whereas a higher initial value indicated a greater likelihood of a poor outcome. Serial testing showed good concordance between changes in SAA titre and clinical course (17).

The consistent elevation of SAA in all tumour types and the more marked elevation in metastatic disease may make its measurement useful in malignancy (18).

- **C-reactive protein (CRP)**

CRP is a protein that is made in the liver and secreted systemically during the process of inflammation in response to the inflammatory cytokine IL-6. This 'acute phase protein' increases when you have certain diseases, which cause inflammation. CRP can be measured in a blood sample. CRP enhanced secretion of IL-6; binds activating Fcg receptors; activates PI3K/Akt, ERK, and NF-kB pathways; and inhibits caspase cascade activation induced by chemotherapy drugs. Further, CRP was shown to synergize with IL-6 in protecting myeloma cells from apoptosis (19).

Blood samples of persons with colon cancer have an average CRP concentration of 2.69 milligrams per litre. Persons without colon cancer average 1.97 milligrams per litre. The difference was statistically significant (11).

A study was conducted to characterize the stage-specific prognostic relevance of preoperative systemic inflammatory response, defined by CRP in colon cancer patients. IN conclusion it was found that an increase of CRP concentrations correlated with clinically relevant poorer disease-specific survival in each stage of colon cancer (27).

In **prostate cancer** elevated CRP levels determined overall survival of patients with castration-resistant prostate cancer treated with docetaxel (20).

Increased levels of CRP together with IL-8 are associated with increase risk of developing **lung cancer** in smokers. The 10-year standardized absolute risks of lung cancer in the PLCO study were highest among current smokers with high IL-8 and CRP levels (absolute risk = 8.01%, 95% CI = 5.77% to 11.05%) (21).

Preoperative elevated CRP levels predict pathological aggressiveness in oral **squamous cell cancer** (22).

The concentrations of IL-6 and CRP were significantly increased in **breast cancer** patients relative to control group and to benign breast tumor group. The frequency of higher levels and absolute value of IL-6 and CRP showed tendency to significant increase with the stage of disease. A positive correlation was observed between the concentrations of IL-6 and CRP. IL-6 and CRP are useful markers for the estimation of progress disease in patients with breast cancer (23).

Elevated CRP and serum amyloid A (SAA) are strongly associated with an increase risk of developing **gastric cancer** in a population-based nested case-control study (24).

Based on a review of ten recent studies, CRP has a critical prognostic value in patients with breast cancer as an inflammation biomarker, that when elevated is strongly predictive of poorer survival in breast cancer (25). The clinical significance of the systemic inflammatory response (SIR) in patients with rectal cancer undergoing neoadjuvant chemoradiotherapy was studied. C-reactive protein is a reliable predictor of recurrence in patients with rectal cancer undergoing chemoradiotherapy followed by surgery (26).

The pleiotropic cytokine IL-6 is a key player in systemic inflammation, regulating both the inflammatory response and tissue metabolism during acute stimulations. High circulating IL-6 levels are correlated with a poor prognosis in breast cancer patients (27).

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3. Glucose and Insulin: Fasting glucose, insulin levels, Hemoglobin A1C, C-Peptide**,** Leptin, and Insulin-like growth factor -1

- **Hyperglycaemia / Hyperinsulinemia**

Hyperglycaemia may be described as an excess of sugar (glucose) in the blood. The endocrine system regulates the amount of sugar that is stored and used for energy. It is important in brain cell function, and energy levels.
Since the sugar that you consume in your diet is either used or stored, certain conditions and disorders may cause you to have difficulty processing and storing blood glucose, resulting in hyperglycaemia or hypoglycaemia. The 1931 Nobel laureate in medicine, German Otto Warburg, Ph.D., first discovered that cancer cells have a fundamentally different energy metabolism compared to healthy cells. The crux of his Nobel thesis was that malignant tumours frequently exhibit an increase in anaerobic glycolysis -- a process whereby glucose is used as a fuel by cancer cells with lactic acid as an anaerobic by-product -- compared to normal tissues.
The large amount of lactic acid produced by this fermentation of glucose from cancer cells is then transported to the liver. This conversion of glucose to lactate generates a lower, more acidic pH in cancerous tissues as well as overall physical fatigue from lactic acid build-up. Thus, larger tumours tend to exhibit a more acidic pH.

This inefficient pathway for energy metabolism yields only 2 moles of adenosine triphosphate (ATP) energy per mole of glucose, compared to 38 moles of ATP in the complete aerobic oxidation of glucose.

The association of hyperglycemia with total cancer risk in women and in women and men combined for several cancer sites, independently of obesity, provides further evidence for an association between abnormal glucose metabolism and cancer. (1)
It has been hypothesised that insulin, a growth factor known for its mitogenic activity,(2) plays a major role in endometrial carcinogenesis. Hypertension and diabetes are markers of insulin resistance/hyperinsulinaemia. (3,4)

Elevated fasting levels of serum **insulin** within the normal range appear to be associated with a higher risk of prostate cancer (5).

IGF-1/IGFBP (insulin-like growth factor binding protein)-3 ratio is important to access in breast cancer patients. Elevated IGF-1 to IGFBP-3 is associated with increase mortality in breast cancer patients (6). Also, **improving vitamin D status** may help lower risk of (colorectal) cancer associated with higher IGF-1/IGFBP-3 ratio or C-peptide levels (7).

Elevated IGFBP-1 in men with metastatic prostate cancer starting androgen deprivation therapy (ADT) is associated with shorter time to castration resistance and overall survival. (9)

Maintaining normal **hemoglobin A1C** (glycated hemoglobin) overall, and good glycemic control can help reduce the burden of cancer. Range <5.8 (8).

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- **C-peptide:** Elevated circulating insulin levels have also been associated with mitogenic and anti-apoptotic effects. C-peptide is a peptide which is made when proinsulin is split into insulin and C-peptide. They split when released from the pancreas and is released into the blood - one C-peptide for each insulin molecule. The reason that the C-peptide levels are measured instead of the insulin levels themselves is because insulin concentration in the portal vein ranges from two to ten times higher than in the peripheral circulation. The liver extracts about half the insulin reaching it (the plasma), but this varies with the nutritional state. The pancreas of patients with type 1 diabetes is unable to produce insulin and they will therefore usually have a decreased level of C-peptide, while C-peptide levels in type 2 patients is normal or higher than normal. There is a strong associated between elevated levels of C-peptide and various cancers including prostate, breast, colon, pancreatic and endometrial (1,2,3,4).

According to a recent study elevated C-peptide was associated with an approximately 50% increased risk of invasive breast cancer. This data suggest a positive association between hyperinsulinemia and breast cancer risk that occurs through nonestrogenic mechanisms, and that is not mediated by breast density (5).

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- **Leptin**

Adipokines (leptin and adiponectin), insulin resistance and sex steroids are associated with breast cancer (1). The adipokine leptin acts through binding to specific membrane receptors. Binding of leptin to its receptor induces activation of different signaling pathways, including the IL-8, JAK/STAT, MAPK, IRS1, and SOCS3 signaling pathways. Expression of leptin and its receptors has been demonstrated to occur in breast cancer cell lines and in human primary breast carcinoma. Leptin is able to induce the growth of breast cancer cells through activation of the Jak/STAT3, ERK1/2, and/or PI3K pathways, and can mediate angiogenesis by inducing the expression of vascular endothelial growth factor (VEGF). In addition, leptin induces transactivation of ErbB-2, and interacts in triple negative breast cancer cells with insulin like growth factor-1 (IGF-1) to transactivate the epidermal growth factor receptor (EGFR), thus promoting invasion and migration. Leptin can also affect the growth of estrogen receptor (ER)-positive breast cancer cells, by stimulating aromatase expression and thereby increasing estrogen levels through the aromatization of androgens, and by inducing MAPK-dependent activation of ER. Taken together, these findings suggest that the leptin system might play an important role in breast cancer pathogenesis and progression, and that it might represent a novel target for therapeutic intervention in breast cancer (2). Leptin also increases the cancer-related growth factors IL-6 (2) and IL-8 (3).

Serum leptin levels and waist-to-hip ratio (WHR) together can help serve as potential prognostic markers, as well as a ETNS target, in metastatic breast cancer (MBC) patients treated with aromatase inhibitors (AIs) (4). Also, the balance of adiponectin and leptin that are the critical factors in breast and other obesity related cancers (5). In a recent study high leptin levels were significantly higher in breast cancer tissue compared with normal tissue (6).

Leptin, in breast cancer is linked to, or regulated, in part by interleukin-1 (IL-1) signaling which in turns upregulates vascular endothelial growth factor (VEGF), promoting angiogenesis.

Higher circulating levels of leptin [OR, 1.94 (1.37-2.75); $P \leq 0.001$), the leptin:adiponectin ratio [OR, 1.91 (1.36-2.68); $P = 0.005$], and CRP [OR, 1.41 (1.01-1.96); $P = 0.014$] are associated with an increased risk of postmenopausal breast cancer (7).

Elevated leptin levels are related to worse prognosis of breast cancer (8).

In prostate cancer leptin appears to be a link between obesity and risk of progression (9).

Serum leptin levels in lung cancer patients were significantly higher compared to those in controls and leptin expression in lung cancer tissue was markedly increased than that in normal lung tissue (both $P<0.050$) (10).

Higher Body Mass Index (BMI) is also associated with greater breast cancer risk and breast cancer progression (11).

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- The hormone ghrelin and the adipocytokines leptin and adiponectin participate in body weight regulation. In response to weight loss, ghrelin and adiponectin levels increase and leptin decreases. Cancer cachexia is a complex metabolic state, characterised by loss of muscle mass and adipose tissue together with anorexia. The hormone ghrelin and the adipocytokines leptin and adiponectin participate in body weight regulation. In response to weight loss, ghrelin and adiponectin levels increase and leptin decreases.

- **Insulin-like growth factor -1**

IGF system changes as prostate tissue progresses from a normal to a malignant state. Differential expression of certain IGF system components in Pca may be associated with the malignant phenotype and more aggressive tumor behaviour. Hence IGFs could serve to predict the outcome of prostatic cancer. (1) The insulin-like growth factor (IGF) ligands stimulate cellular proliferation and survival by activating the type I insulin-like growth factor receptor (IGF-IR). As a result, the IGF signalling system is implicated in a number of cancers, including those of the breast, prostate, and lung. (2)

Cancer cachexia is a complex metabolic state, characterized by loss of muscle mass and adipose tissue together with anorexia. (3) There is a link between leptin, a cytokine that is elevated in obese individuals, and cancer development and can act as a mitogen and an angiogenic factor. (4) Colorectal cancer risk increased with increasing levels of C-peptide. Chronically high levels of circulating insulin and IGFs associated with a Western lifestyle may increase colorectal cancer risk, possibly by decreasing IGFBP-1 and increasing the bioactivity of IGF-I. (5)

Hemoglobin A1c (HbA1c) level at a baseline examination was an independent predictor of new onset diabetes. Levels of fasting glucose, HbA1c, insulin requirements, total cholesterol, HDL, LDL, triglycerides, fibrinogen, PAI-1, tPA and C-reactive protein in men with insulin-dependent diabetes, androgen deprivation therapy may have negative effects on their glycaemic control and may aggravate the biochemical risk profile of cardiovascular disease. (6)

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4. Vitamin D (25 OH and di1,25H)

Vitamin D is not truly a "vitamin," but rather a substrate (non-steroidal hormone) for a potent, pleiotropic (meaning it produces multiple effects), repair and maintenance seco-steroid hormone that serves multiple gene-regulatory functions in your body. So far, about 3,000 genes have been found that are regulated by vitamin D. (1)

32% of doctors and med school students are vitamin D deficient.
40% of the U.S. population is vitamin D deficient.
48% of young girls (9-11 years old) are vitamin D deficient.
Up to 60% of all hospital patients are vitamin D deficient.
76% of pregnant mothers are severely vitamin D deficient, causing widespread vitamin D deficiencies in their unborn children, which predisposes them to type 1 diabetes, arthritis, multiple sclerosis and schizophrenia later in life. 81% of the children born to these mothers were deficient.
Up to 80% of nursing home patients are vitamin D deficient. (2)
Populations at high risk for deficiency include the elderly, adolescents, people with darker skin, those who are obese, and those with limited sun exposure. Studies have shown that a deficiency cannot only lead to bone disease, but possibly cancer, cardiovascular disease and autoimmune diseases. (3) More than three-quarters of cancer patients have insufficient levels of vitamin D (25-hydroxy-vitamin D) and the lowest levels are associated with more advanced cancer. Research shows how vitamin D may impact specific features of cancer, such as the stage or extent of tumour spread, prognosis, recurrence or relapse of disease, and even sub-types of cancer. (4) Vitamin D deficiency has been linked to everything from cancer and heart disease to diabetes. (5)

Optimizing your vitamin D levels can help you to prevent as many as 16 different types of cancer including pancreatic, lung, breast, ovarian, prostate, and colon cancers, as well as heart disease (6), diabetes, neurological disease (8), autoimmune disease (9), osteoporosis (10), depression (11) and more. Higher concentrations of 25(OH)D (>50 and <100) were inversely associated with all-cause and CVD mortality among adults with hypertension in the US. (12) Even if you have cancer, by taking vitamin D you can increase your over-all survival. (13, 14,15) Maintaining healthy normal levels of **Vitamin D can significantly lower your risk of dying from any cause**. (16, 17)

Sufficient baseline vitamin D levels is associated with better prognosis and predictive of Zometa (zoledronic acid) benefit among postmenopausal women (18)

Vitamin D-3 dosage must be adjust to the individual and based on blood-work assessment of both the 25 OH (25-hydroxy-vitamin D) and 1,25 diH (1,25 dihydroxy-vitamin D) Vitamin D levels. Optional levels are between 50-80. In the kidney, 25-hydroxy vitamin D changes into an active form (1,25-dihydroxy vitamin D). The active form helps control blood levels of calcium and phosphate. Vitamin K should always be included with Vitamin D supplementation. People with autoimmune conditions or lymphoma are particularly prone to hypercalcemia from taking high amounts of vitamin D. Darker skin people living in the north, and/or during the winter often need to take higher levels of vitamin D. It is important to either add Vitamin K (150 – 300 mcg.) or use a Vitamin D supplement with vitamin K.

Many people have Vitamin D receptor polymorphisms, which makes them more prone to several diseases including cancer, and these people often need higher amounts of supplemental vitamin D to achieve optimal levels.

I have found people need about twice as much vitamin D in the winter than in the summer, and in some people that get regular sun (20 minutes per day 3-5 days a week, in short sleeves and shorts) often don't need to supplement as all during the summer.

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5. Copper, Ceruloplasmin and Zinc

Copper is believed to be the switch that turns on the angiogenesis process in tumour cells. It has been observed that abnormally high serum copper levels are found in patients with many types of progressive tumours. (1) The University of Michigan reported that researchers had "successfully stopped the growth and spread of cancer by depriving the tumours of the copper supply they need to form new blood vessels. (2) Copper chelation reduces the secretion of many angiogenic factors and reduces tumour growth and microvascular density in animal models. (3) People with higher serum iron, transferrin saturation (TS), or copper concentrations had an increased risk of dying from cancer. (4)

Overall incidence of cancer was positively associated with serum ceruloplasmin level. The association was strongest for lung cancer and other cancers related to smoking and, consequently, in males. (5)

Serum ceruloplasmin increases in cancer patients and appears be a reliable marker for prostate, (6,7) colon (7) and other cancers.

Ceruloplasmin together with copper and zinc, can be used for monitoring Hodgkin's Disease (8), as well as NHL.

Serum concentration of copper was significantly higher in patients with breast cancer in a recent study. Alteration in the concentration of copper and zinc in serum of patients with breast cancer, which may indicate abnormal copper and zinc metabolism in females with breast cancer. (9)

In cervical cancer ceruloplasmin can serve as a potential predictive biomarkers for the progression of high grade lesions (10).

Serum zinc may be good prognostic indicator in locally advanced cancer cervix patients receiving chemo-irradiation. A study evaluated the prognostic significance of serum Zinc (S.Zn) in locally advanced cancer cervix (LACC) patients treated with chemo-irradiation. After completion of treatment, the patients were divided into 2 groups based on the response, 1 - Complete response (CR) and 2 - Partial/No response (PR/NR). These groups were compared based on the observations of the studied parameter. The mean post-treatment S.Zn values were significantly higher in group 1 as compared to group 2 ($P < 0.05$). Patients achieving CR had higher mean levels of S.Zn compared to patients achieving PR/NR (11).

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6. Ferritin, soluble transferrin receptor, Transferrin Saturation

Iron transport in plasma is carried out by transferrin, which donates iron to cells through interaction with a specific membrane receptor. (1) It is well known that iron plays an essential role in many biochemical reactions and that rapidly growing cells require more iron for their growth and metabolism than resting cells. Transferrin and its receptor are required for entry of iron into the cell. In contrast, ferritin is a cellular storage protein whose main function is to sequester excess ferric iron and thus prevent high concentrations of soluble ferric iron from becoming toxic to the cell. The quantitation of expression of transferrin receptor and ferritin heavy chain mRNA may be useful for assessing prognosis and guiding therapeutic decisions in breast cancer patients. (2)
Soluble transferrin receptor (sTfR) and ferritin concentrations were measured in a variety of clinical settings to compare the ability of these two tests to identify iron deficiency. Iron-deficient patients with diagnoses that included systemic lupus erythematosus, adenocarcinoma of the lung, chronic liver disease, colon cancer. (3) Data points to a central role for the cytokine network in the modulation of iron metabolism in the acute-phase response and anaemia of chronic disease. TNF, possibly via induction of IL-6, and IFN-γ induce hypoferraemia, which may in part result from a decrease in tissue iron release based on a primary stimulation of ferritin synthesis. The fall in sTfR levels may reflect an impaired erythroid growth and/or TfR expression mediated by TNF and IFN-γ. (4)

Iron homeostasis in normal cells is accurately balanced and tightly regulated, in cancer cells, however, this balance is frequently and consistently compromised (5). Several recent reports have suggested that an association may exist between altered intracellular iron homeostasis, perturbations in the functioning of proteins involved in the iron-regulatory pathways, and breast cancer. (6) A combination of high ferroportin and low hepcidin expression is associated with poor outcomes in breast cancer. (7)

The peptide hormone hepcidin plays a central role in regulating dietary iron absorption and body iron distribution. Many human diseases are associated with alterations in hepcidin concentrations. The measurement of hepcidin in biological fluids is therefore a promising tool in the diagnosis and management of medical conditions in which iron metabolism is affected. The measurement of hepcidin in biological fluids is therefore a promising tool in the diagnosis and management of medical conditions in which iron metabolism is affected. (9) Hepcidin as a promising diagnostic tool and therapeutic target for iron disorders. It is an acute-phase reactant, responding to infection and inflammation. (8)

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7. **Methylation: Homocysteine, MTHFR SNPs**

Increased total homocysteine (t-Hcy), decreased total glutathione (t-GSH) and folate levels were observed in lung cancer patients compared with healthy controls. Homocysteine is suggested, as a marker for several types of cancer but further investigation in the larger cancer population would clarify the importance of homocysteine as a cancer marker. (1) Malignant cells are characterised by high a growth rate, and the methionine requirement increases in these cells due to increased protein synthesis and transmethylation reactions. Normal cells meet their methionine requirement by synthesizing it from homocysteine. In contrast, methionine-dependent malignant cells in organs such as the lung, kidney, breast, colon and bladder cannot convert homocysteine to methionine, which results in homocysteine accumulation. (2) Studies showed that patients with ovarian (3), pancreatic (4), colorectal (5), head and neck squamous cell carcinomas (6,7) and acute lymphoblastic leukaemia (8) had higher homocysteine levels

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8. **Endocrine-related factors:** Estradiol, SHBG, Estrone Sulfate, 2:16[alpha]-Hydroxyestrone Ratio, Prolactin, DHEA sulphate, Male panel: Testosterone (Total, free & DHT)

• **Estradiol (E2)** Circulating estrogen levels have been shown to correlate with the expression of classically estrogen responsive genes in ER+ breast tumors, suggesting that E2 (estradiol) measurements may prove to be a valuable marker for endocrine sensitivity and for refining individualized treatment regimes (1,2,3).

Plasma estrogens show the strongest correlations with risk and these are strengthened by measurement or calculation of the proportion of estradiol that circulates free of sex hormone binding globulin (SHBG), consistent with this being the most active fraction. The relationships have been reported to potentially explain virtually all of the association of breast cancer with body mass index in postmenopausal women; this is likely to be due to non-ovarian estrogen synthesis being prominent in subcutaneous fat. These strong relationships have led to plasma and urine estrogen levels being used as intermediate end-points in the search for genes that affect breast cancer risk via their role in steroid disposition. Plasma androgen levels also show a relationship with breast cancer risk that is weakened but not eliminated by 'correction' for estrogen levels. This has been argued to be evidence of the local production of estrogens being important in the etiology of breast cancer. Given that plasma steroid levels do not correlate closely with mammographic density, which is strongly associated with risk, the opportunity exists to combine the two factors in assessing breast cancer risk but the low availability of suitable estrogen assays is a major impediment to this. In established breast cancer, plasma estrogens have been found to correlate with gene expression of estrogen dependent genes and the expression of these varies across the menstrual cycle of premenopausal women. There is infrequently a need for routine measurement of plasma estrogen levels but it has been important in the comparative pharmacology and dose-related effectiveness of aromatase inhibitors. Measurement may be needed to identify residual ovarian function in women who have amenorrhea subsequent to cytotoxic chemotherapy indicating their unsuitability for aromatase inhibitor treatment. Use of highly sensitive assays has also revealed that the association between BMI and plasma estrogen levels persists in patients on 3rd generation aromatase inhibitors and that measurable increments in plasma estrogen levels occur with some vaginal estrogen preparations that are of concern in relation to treatment efficacy (4).

Nine prospective case-control studies conducted between 1990 and 2000 accrued evidence for this relationship between plasma estrogens and risk. An overview pooling the data from the studies revealed that there was an overall increase in relative risk of 1.29 (95% CI, 1.15 to 1.44; P_.001) for every doubling of E2 concentration (5,6,7,8,9,10,11,12,13).

There is evidence suggesting estrogen levels may not be fully suppressed by Aromatase Inhibitors (AIs) treatment in some obese women despite effective aromatase inhibition (14,15). The mechanism causing this effect is currently being investigated (16,17).

Single nucleotide polymorphisms (SNPs) in the gene encoding aromatase (CYP19A1) have been associated with differential benefit from letrozole treatment in metastatic breast cancer (mBC) patients (18) “AIs exert antiproliferative effects by reducing local estrogen production from androgens in postmenopausal women with hormone-responsive breast cancer. Androgen metabolites generated by the aromatase-independent enzymes, 5α-androstane-3β, 17β-diol (3β-diol), androst-5-ene-3β, and 17β-diol (A-diol), also activate estrogen receptor (ER) α. Estradiol (E2) can also reportedly be generated from estrone sulfate (E1S) pooled in the plasma. Estrogenic steroid-producing aromatase-independent pathways have thus been proposed as a mechanism of AI resistance. However, it is unclear whether these pathways are functional in clinical breast cancer. To investigate this issue, we assessed the transcriptional activities of ER in 45 ER-positive human breast cancers using the adenovirus estrogen-response element-green fluorescent protein assay and mRNA

expression levels of the ER target gene, progesterone receptor, as indicators of ex vivo and in vivo ER activity, respectively. We also determined mRNA expression levels of 5α-reductase type 1 (SRD5A1) and 3β-hydroxysteroid dehydrogenase type 1 (3β-HSD type 1; HSD3B1), which produce 3β-diol from androgens, and of steroid sulfatase (STS) and 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD type 1; HSD17B1), which produce E2 or A-diol from E1S or dehydroepiandrosterone sulfate. SRD5A1 and HSD3B1 expression levels were positively correlated with ex vivo and in vivo ER activities. STS and HSD17B1 expression levels were positively correlated with in vivo ER activity alone. Elevated expression levels of these steroid-metabolizing enzymes in association with high in vivo ER activity were particularly notable in postmenopausal patients. Analysis of the expression levels of steroid-metabolizing enzymes revealed positive correlations between SRD5A1 and HSD3B1, and STS and HSD17B1. These findings suggest that the SRD5A1-HSD3B1 as well as the STS-HSD17B pathways, could contributes to ER activation, especially postmenopause. These pathways might function as an alternative estrogenic steroid-producing, aromatase-independent pathways"(19).

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- **Sex-hormone binding globulin (SHBG)**

Sex hormone-binding globulin (SHBG) not only regulates the free concentration of certain steroid sex hormones in plasma, but is involved in a nongenomic mechanism of steroid hormone action. Intracellular cAMP causes increased growth in this prostate cancer cell line, and that both SHBG-dihydrotestosterone and SHBG-oestradiol can regulate intracellular cAMP, and hence growth, in these cells. (1) The risk for breast cancer increased statistically significantly with increasing concentrations of all sex hormones examined: total estradiol, free estradiol, non-sex hormone-binding globulin (SHBG)-bound estradiol (which comprises free and albumin-bound estradiol), estrone, estrone sulfate, androstenedione, dehydroepiandrosterone, dehydroepiandrosterone sulfate, and testosterone. (2)
Insulin and prolactin (PRL) inhibit SHBG production and confirm that T4, T, and E2 stimulate SHBG production in vitro. These findings suggest that insulin and PRL may be important factors in the regulation of SHBG production in vivo. (3)

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- **Estrone sulfate**

Estrone sulfate (E1S) is one of the most important and underutilized hormone measurements. Quantitatively it is the most important circulating estrogen (1). E1S represents a slowly metabolized estrogen reservoir. Sulfated hormones have a 10-fold lower clearance rate than glucuronides (2). The metabolic clearance rate of estrone sulfate is the lowest by far of any estrogen. Levels of estrogens and their sulfates are many times higher in breast cancer tissue than in blood (3). In fact the ratio of E1S to estrone is significantly higher in breast and endometrial cancer (4,5). This ratio could provide valuable breast cancer risk information (6). Increases in serum E1S levels during menopausal hormone therapy are associated with increases in mammographic density (7).

- Note: Many physicians are at times puzzled when the administration of estrogens has not lead to an elevation in either estradiol or estrone. Consequently many practitioners simply increased

the dosage of the estrogens prescribed. This promoted excess estrogen related side effects, furthering the confusion. However, when the E1S was measured, it was extremely high.

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- **2:16[alpha]-Hydroxyestrone Ratio**

Experimental and clinical evidence suggests that 16α-hydroxylated oestrogen metabolites, biologically strong oestrogens, are associated with breast cancer risk, while 2-hydroxylated metabolites, with lower oestrogenic activity, are weakly related to this disease (1,2).

Reference:

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- **Prolactin**

Prolactin can promote diverse carcinomas in mice (1). Substantial epidemiological, clinical, and biological evidence now exists confirming that prolactin promotes human breast cancer (2). Patients have responded to L-dopa with relief of bone pain and a 50% decrease in serum prolactin. Near-zero prolactin levels following hypophysectomy in some patients have been correlated with clinical remissions (3).

Reference:

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- **DHEA Sulfate**

The ability of MCF-7 human breast cancer cells to hydrolyse extracellular oestrone (E1S) and dehydroepiandrosterone (DHEAS), indicate that these reactions are mediated by different enzymes, and demonstrate that DHEAS is a potent inhibitor of MCF-7 E1 sulfatase. Circulating DHEAS, therefore, may substantially limit the ability of most postmenopausal breast cancers to use E1S as a substrate for intracellular oestrogen biosynthesis. (1) Results suggest that adrenal androgens are positively associated with breast cancer among predominately premenopausal women, especially for oestrogen receptor–positive/progesterone receptor–positive tumours and among women over age 45 years. Both DHEA and DHEAS were positively associated with oestrogen receptor–positive/progesterone receptor–positive breast cancer. (2)

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- **Male Hormone panel**

SHBG, testosterone (total, free, and dihydrotestosterone (DHT), prolactin, and oestrodial
There is not now—nor has there ever been—a scientific basis for the belief that testosterone (T) causes Prostate Cancer (pCA) to grow. Discarding this modern myth will allow exploration of alternative hypotheses regarding the relationship of T and pCA that may be clinically and scientifically rewarding. (1) However, administration of T and other steroids to men suspected of harbouring pCA, at this time, should be considered only as part of properly sponsored, well designed, organised, rigidly controlled, and carefully monitored clinical trials. (2) There are theoretical concerns that testosterone treatment of older men could increase clinical prostate cancer; however, available evidence is not convincing. (3) The prevalence of symptomatic hypogonadism in male patients with cancer exceeds that found in comparable studies in non-cancer populations. (4) Cachexia patients have higher inflammation and lower testosterone, grip strength, functional status, erectile function, fat mass, and appendicular lean body mass. Inflammation, total testosterone (TT), and albumin are associated with heavier symptom burden in this population. (5)

Recurrent prostate cancer may develop the capacity to biosynthesize testicular androgens from adrenal androgens or cholesterol. This surprising finding suggests intracrine production of dihydrotestosterone. (6) The wide range of variation for T in patients with carcinoma would suggest that although mean T is higher in these patients, this measurement alone is of little practical value, whereas T/DHT ratio is a more reliable parameter in evaluating the androgen changes in these patients. (7) Results indicate that elevated serum testosterone, together with decreased serum oestrogens, may promote the development of hepatocellular Carcinoma (HCC). (8)

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- **Thyroid panel**

Subclinical hyperthyroidism increases risk of certain solid tumours and that spontaneous hypothyroidism may delay onset or reduce aggressiveness of cancers. Accumulating clinical evidence may justify new, broadly-based controlled studies in cancer patients of the possible contribution of thyroid hormone to tumour behaviour. (1) Thyroid hormones (TH) have been also implicated in cellular transformation, tumourigenesis and metastasis, assuming particular importance in tumour induced angiogenesis. TH-induced angiogenesis is thought to be initiated at integrin αvβ3 membrane receptor mainly through T_4 binding. Therefore, targeting TH actions could be an alternative adjuvant therapy against cancer proliferation and angiogenesis. (2) In ERα-positive (estrogen receptor-α) human lung cancer cells, the proliferative action of thyroid hormone initiated at the plasma membrane is at least in part mediated by ERα. In summary, thyroid hormone may be one of several endogenous factors capable of supporting proliferation of lung cancer cells. (3)

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9. Markers of Angiogenesis

Vascular Endothelial Growth Factor (VEGF), IL-8, LDH, Lactate, cathepsin B, Survivin, MMP-2,3 and 9, HIF-1a

Compelling experimental and clinical data support the concept that breast carcinoma, as most of the other solid tumours, needs to develop the angiogenic phenotype for invasiveness, progression and metastasis. Certain angiogenic peptides have been assessed in human breast cancer: vascular endothelial growth factor (VEGF), platelet derived-endothelial cell growth factor (PD-ECGF, also known as thymidine phosphorylase, TP) and fibroblast growth factor family (FGFs). Among these, the most studied is VEGF, which appears to be a powerful prognostic indicator. (1) Survivin is the smallest member of the inhibitor of apoptosis (IAP) gene family and is involved in angiogenesis. (2) the immunoreactivity of survivin increased in the transition from adenoma with low dysplasia to high dysplasia/carcinoma, which was associated with a decrease in tumour cell apoptosis and increases in cell proliferation and angiogenesis during colorectal tumourigenesis. (3) Vascular endothelial growth factor (VEGF) is an endothelial cell mitogen and permeability factor that is potently angiogenic and the angiogenic activity attributed to VEGF may be due in part to its ability to enhance endothelial cell survival by inducing expression of Bcl-2. (4)

Plasma VEGF-A and VEGFR-2 are predictive markers for Avastin (bevacizumab) efficacy in metastatic breast cancer, supporting findings in gastric and pancreatic cancers. (5)

Expression of VEGF and VEGF 1 and 2 is associated with invasive breast cancer: prognostic significance and relationship with markers for aggressiveness (6).

As a result of deprivation of oxygen (hypoxia) and nutrients, the growth and viability of cells is reduced. Hypoxia-inducible factor (HIF)-1a helps to restore oxygen homeostasis by inducing glycolysis, erythropoiesis and angiogenesis. (7)

Lactate dehydrogenase 5 (LDH-5) regulates, under hypoxic conditions, the anaerobic transformation of pyruvate to lactate for energy acquisition. Several studies have shown that serum LDH may be an ominous prognostic marker in malignant tumours. (8)

VEGF and matrix metalloproteinase (MMP)-9VEGF and MMP-9 may be potential biomarkers for the diagnosis and prognosis of non-small cell lung cancer (NSCLC). The present study was designed to detect

the serum levels of VEGF and MMP-9 in NSCLC, and to explore their diagnostic and prognostic values. A total of 543 cases were involved, of which 332 were NSCLC (272 cases in the pretreatment group and 60 cases in the postoperative group), 91 were patients with benign lung diseases and 120 were healthy controls. The serum levels of VEGF and MMP-9 were found to be significantly higher in the pretreatment group than those in the patients with benign lung diseases and healthy controls (VEGF, $P<0.0001$; MMP-9, $P<0.0001$). Compared with the pretreatment group, the serum levels of VEGF and MMP-9 in the postoperative group were significantly decreased (VEGF, $P=0.005$; MMP-9, $P=0.002$), and the levels of VEGF and MMP-9 in the pretreatment group of patients with stages III and IV were higher than those with stages I and II (VEGF, $P<0.0001$; MMP-9, $P=0.021$). In addition, the levels of VEGF and MMP-9 were found to closely correlate with lymph node metastasis (VEGF, $P<0.0001$; MMP-9, $P<0.0001$) in the pretreatment group, while being independent of other clinicopathological parameters ($P>0.05$). Furthermore, a positive correlation was observed between the serum levels of VEGF and MMP-9 ($r=0.159$; $P=0.009$). Additionally, the serum levels of VEGF and lymph node metastasis were identified as independent prognostic factors of the inoperable NSCLC patients in a multivariate Cox regression analysis ($P<0.05$). (9)
Serum levels of vascular endothelial growth factor are increased and correlate with malnutrition, immunosuppression involving MDSCs and systemic inflammation in patients with cancer of the digestive system. (10)

Interleukin 8 (IL-8) is a proinflammatory cytokine biomarker associated cancer angiogenesis. IL-8 promotes cell growth and migration and contributes to aggressive cancer phenotypes. (11, 12, 13) Serum levels of IL-8 are consistently elevated in patients with recurrent or metastatic cancer. (14) Recently, IL-8 has demonstrated a promotional effect on breast cancer invasion and angiogenesis. (15, 16), Serum IL-8 level is useful in determining metastatic breast cancer. (17) Also, The depletion of IL-8 causes cell cycle arrest and increases the efficacy of docetaxel in breast cancer. (18)
Avastin (bevacizumab) is a monoclonal antibody to VEGF-A. In head and neck squamous cell carcinoma IL-8 appears induces resistance to avastin. Downregulation of IL-8 resulted in sensitization of resistant tumors to Avastin by disrupting angiogenesis and enhancing endothelial cell apoptosis. (19)
Elevated **IL-8**, TNF-α, and MCP-1 in men with metastatic prostate **cancer** starting androgen-deprivation therapy (ADT) are associated with shorter time to castration-resistance and overall survival. (20)

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prostate **cancer** starting androgen-deprivation therapy (ADT) are associated with shorter time to castration-resistance and overall survival. Prostate. 2014 Mar 26. doi: 10.1002/pros.22788.

- **Matrix metalloproteinases (MMPs)** are frequently expressed in malignant tumors and play an important role in tumor invasion and metastasis. MMP-2 and MMP-9 expression has been correlated with angiogenesis and poor survival in some tumors (1).

MMP-2 and -9 are novel biomarkers of patients with metastatic breast cancer (2). Elevated levels of MMP-2 often correlate with the up regulation of VEGF of the tumor (3). Serum level of MMP-2 is closely correlated with blood micrometastasis. And its high level may be an important contributing factor for the metastasis of breast cancer (4).

MMP-2 or **MMP-9**, serves as a useful statistically independent prognostic factor in ovarian cancer, thus helping to identify ovarian cancer patients with an aggressive form of the disease (5).

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- **Hepatocyte Growth Factor (HGF)** causes many biological events, including cell proliferation, movement, invasiveness, morphogenesis, and angiogenesis. Elevated HGF content in tumor tissue was reported to predict a more aggressive biology in non–small cell lung cancer patients.

Mesenchymal-epithelial transition (MET) is a member of a distinct subfamily of heterodimeric receptor tyrosine kinase receptors that specifically binds the HGF. High levels of HGF and/or cMET correlate with poor prognosis in several tumor types, including breast, ovarian, cervical, gastric, head and neck, and non-small-cell lung cancers (1).

Breast cancer patients with more advanced tumor node-metastasis staging were shown to have higher serum soluble HGF. Thus, preoperative serum soluble HGF levels might reflect the severity of invasive breast cancer and deserve further evaluation (2)

MET activation drives the malignant progression of several tumor types, including colorectal cancer (CRC), by promoting signaling cascades that mainly result in alterations of cell motility, survival, and proliferation (3).

Synchronous liver metastasis (SLM) remains a significant problem in newly diagnosed colorectal cancer. Expression of HGF and Met at the protein level and the RNA level in primary CRCs with SLM were significantly higher than that in primary colorectal carcinomas without liver metastases

(4).
HGF autocrine glioblastoma bears an activated MET signaling pathway that may predict sensitivity to MET inhibitors (5).

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10. Serum HER II neu (HER II+ cancer)
One of the four transmembrane receptors that belong to the erB family, is the HER II neu oncoprotein. It forms heterodimers by binding to specific ligands, enhancing cell signaling and assisting in cell growth and differentiation. Serum HER II neu is a biomarker that is able to predict patients that response or don't respond to HER II targeted therapy, such as Herceptin and/or Tykerb (1).

Previous reports suggested that changes in serum HER-II neu levels appear to predict response or lack of response to Herceptin-based therapies in metastatic breast cancer (2,3).
There is a high statistical correlation between IHC pathology+ HER II cancer and elevated serum levels. Serum HER II neu cut-off level was 18.4ng/ml; 46.7% of patients were serum HER2-positive and 43% were IHC positive (4).
Elevated baseline sHER II levels (>15 ng ml^{-1}) and a decrease of sHER2 levels (>20%) early after therapy initiation are both relevant criteria to predict response to lapatinib (Tykerb)-based treatment (5).
High EGFR expression is associated with decreased adjuvant herceptin benefit in HER-II invasive breast cancer; however those patients would most likely benefit from tykerb because it targets both HER-II and EGFR, also sometimes called HER-I (6).
Serum HER-2/neu and EGFR may represent useful markers for early prediction of probability of response, PFS, and OS in patients with advanced breast cancer treated with metronomic chemotherapy (7, 8).
In a study involving 107 breast cancer patients serum HER2 levels >30 ng/ml were identified to correlate with increased risk of brain metastases (9).

The Predictive Value of Serum HER2/neu for Response to Anthracycline-Based and Trastuzumab-Based Neoadjuvant Chemotherapy. Serum HER2/neu levels during treatment were associated with pathologic response in patients receiving neoadjuvant chemotherapy, particularly, in a trastuzumab-based regimen. The change of serum HER2/neu levels may serve in monitoring neoadjuvant therapy in HER2/neu-overexpressed breast cancer (10).

Profiling serum HER-2/NEU in prostate cancer

HER2/neu plays a major role in understanding the oncogenesis of prostate adenocarcinoma. For this reason, clarifying the HER2/neu expression is particularly important in androgen independent prostate cancer (PCa), due to the increasing interest in using anti-HER2 targeted therapies for advanced disease treatment. On the other hand, the overexpression of HER2/neu has been reported to release soluble extracellular domain (ECD) in the serum of PCa patients. For this reason, the present review focuses only on studies referring to Serum HER2/neu levels in PCa patients. Serum levels of HER2/neu generally increase with advanced disease state and higher levels have been associated with recurrent or metastatic PCa and a clinically worse outcome. Therefore, it may be concluded that since there is a correlation between increased HER2/neu levels and a poor prognosis in prostate adenocarcinoma, serum HER2/neu could be used in clinical practice and follow up of patients with advanced PCa (11).

Increased risk of cause specific death in PCa patients with serum HER2/neu above the cut off values

Reference	HER2/neu cut off values	p value
Osman I, 2005[51]	14ng/ml	<0.001
Domingo-Domenech J, 2008[41]	15ng/ml	<0.0001

Association between serum HER2/neu (ELISA) and its tissue expression (IHC, FISH) in PCa

Reference	No PCa patients	HER2/neu overexpression- IHC positive	ELISA vs IHC (p value)	HER2/neu amplification-FISH
Lara PN, 2002[37]	62	8%	>0.05	Negative
Domingo-Domenech J, 2008[41]	69	35.3%	0.016	Negative
Tambo M, 2009[49]	75	24%*	>0.05	NA

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11. LDH and Lactate (measures cellular pH alteration, an influence of the cancer (glycolytic) metabolism and hypoxia), CA-9, and uric acid

Over seven decades ago, classical biochemical studies indicated that tumours altered metabolic profiles, displaying high rates of glucose uptake and glycolysis. Although these metabolic changes are not the fundamental defects that cause cancer, they might confer a common advantage on many different types of cancers, which allows the cells to survive and invade. (1) Lactate dehydrogenase-5 (LDH-5) catalyses the reversible transformation of pyruvate to lactate, having a principal position in the anaerobic cellular metabolism. Induction of LDH-5 occurs during hypoxia and LDH-5 transcription is directly regulated by the hypoxia-inducible factor 1 (HIF1). Overexpression of LDH-5 is a common event in non-small-cell lung cancer, can be easily assessed in paraffin-embedded material and provides important prognostic information, particularly when combined with other endogenous markers of hypoxia and acidity. (2) Uric Acid (UA), at physiologic concentrations, has profound effects on human vascular cells. UA alters the proliferation/migration and nitric oxide (NO) release of human vascular cells, mediated by the expression of C-Reactive Protein (CRP) (3)

Lactic acidosis is a well-recognized problem in patients with hyper-leucocytic leukemia (4,5) and in lymphomas (6) and reflects the high metabolic rate of the tumour cells and the associated tissue hypoxia due to hyper-leucocytosis. In solid tumors, however, simultaneous liver involvement appears to be an important determinant causing impairment of lactic metabolism. (7-10) Lactic acidosis has been previously reported in association with malignancy, usually leukemia or lymphoma, (11-13) also in solid tumors. (14-16) Lactic acid is an important energy source for tumor cells and it has been known for more than 50 years that low-oxygen, or hypoxic, cells cause resistance to radiation therapy. (17,18) The ability of cancer cells to resist glucose deprivation-induced cell death is conferred, at least in part, by lactic acidosis, and it is envisioned that disrupting the lactic acidosis may resume the sensitivity of cancer cells to glucose deprivation. (16) The metabolic environment of the tumour is directly affected by lack of vasculature and subsequent oxygen starvation. Hypoxia in the microenvironment results in acidosis, as lactic acid builds up due to anaerobic glycolysis. Abnormally high lactate production in these subjects, perhaps related to the metabolism of the cancer itself. Studies during the presence of lactic acidosis, and again later when the lactate level was lower, showed about twice as much recycling after acidosis was alleviated, suggesting inhibition of hepatic glucose production from lactate during acidosis. It is suggested that the potential for lactic acidosis exists in patients with widespread progressive tumor growth. (19)

The acidic environment inhibits the efficacy of more alkaline chemotherapeutic drugs. Hypoxic conditions also appear to promote tumor survival and growth by creating genomic instability and selecting for more aggressive phenotype cells. (20,21)

Measuring lactic acid shows the amount of lactate (lactic acid) in blood. (22) This test may be done for suspected conditions such as hypoxia (lack of blood and oxygen) to a patient. Two text-books offer greater detail into the acid/alkaline balance. (23,24)

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12) Markers of redox/anti-oxidant status: Glutathione (GSH), Selenium, Malondialdehyde (MDA), and Carotenoid levels

- **Glutathione**

Glutathione is an abundant natural tripeptide found within almost all cells. Glutathione is highly reactive and is often found conjugated to other molecules via its sulfhydryl moiety. It instils several vital roles within a cell including antioxidation, maintenance of the redox state, modulation of the immune response and detoxification of xenobiotics. With respect to cancer, glutathione metabolism is able to play both protective and pathogenic roles. It is crucial in the removal and detoxification of carcinogens, and alterations in this pathway, can have a profound effect on cell survival. However, by conferring resistance to a number of chemotherapeutic drugs, elevated levels of glutathione in tumour cells are able to protect such cells in bone marrow, breast, colon, larynx and lung cancers. (1) The glutathione (GSH) content of cancer cells is particularly relevant in regulating mutagenic mechanisms, DNA synthesis, growth, and multidrug and radiation resistance. In malignant tumours, as compared with normal tissues, that resistance associates in most cases with higher GSH levels within these cancer cells. Experimental evidence shows that acceleration of GSH efflux facilitates selective GSH depletion in metastatic cells. (2) The role of glutathione (GSH) and related enzymes in cellular resistance to xenobiotics, including chemotherapy is well established. (3)
Various natural carotenoids, besides beta-carotene, were proven to have anticarcinogenic activity, and some of them showed more potent activity than beta-carotene. Thus, these carotenoids (agr-carotene, lutein, zeaxanthin, lycopene, rbeta beta-cryptoxanthin, fucoxanthin, astaxanthin, capsanthin, crocetin and phytoene), as well as beta-carotene, may be useful for cancer prevention. (4)

- **Selenium**

Many prospective epidemiological studies and empirical research as well have shown an association between a low prediagnostic serum selenium concentration and the risk of cancer (5, 6). Two studies have found greater concentrations of selenium were associated with reduced prostate cancer risk (7, 8).
The potential anticarcinogenic actions of selenium, an essential trace mineral, are in part related to its antioxidant properties, which is a function of its role in maintaining the enzyme glutathione peroxidase. Selenium is an essential part of the enzyme glutathione peroxidase that neutralizes or catabolizes peroxides to prevent the formation of free radicals that cause oxidative damage. When present in high doses, selenium has also been shown to suppress cell proliferation and enhance immune response. Supplementation of selenium during the promotion phase of cancer significantly decreased the DNA damage and has an overall protective on cells and organs such as the liver (9).

- **Malondialdehyde (MDA)**

Elevated levels of MDA are associated with oxidative stress, and MDR is specifically a marker of lipid peroxidation (10), in patients and is a prognostic indicator for breast, lung (11,12, 13) and prostate cancer (13).

In all stages, MDA levels in total breast cancer patients were significantly higher than those in healthy subjects (14).

Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking (15).

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13) Circulating Tumor Cells (CTCs)

Metastases from primary tumours are responsible for most cancer deaths. It has been shown that circulating tumor cells (CTCs) can be detected in the peripheral blood of patients with a variety of metastatic cancers and that the presence of these cells is associated with poor clinical outcomes. (1) Patients in a training set with levels of circulating tumour cells equal to or higher than 5 per 7.5 ml of whole blood, as compared with the group with fewer than 5 circulating tumour cells per 7.5 ml, had a shorter median progression-free survival (2.7 months vs. 7.0 months, $P<0.001$) and shorter overall survival (10.1 months vs. >18 months, $P<0.001$). The number of circulating tumour cells before treatment is an independent predictor of progression-free survival and overall survival in patients with metastatic breast cancer. (2) Viable tumour-derived epithelial cells (circulating tumour cells or CTCs) have been identified in peripheral blood from cancer patients and are probably the origin of intractable metastatic disease. (3,4) CTC that exhibit specific molecular characteristics including stem cell characteristics, could be able to create new metastasis. Hormone therapy or anti-erbB2 therapies are prescribed according to the hormone (ERα/PR expression) and erbB2 status of the initial tumor. Nonetheless, it appears that the CTC, and consequently the metastatic cells, may have a very different hormone and erbB2 status. An optimal individualized treatment could then be obtained by characterizing ERα and erbB2 status in the CTC and comparing it to the primary tumor (5). Evaluation of isolated tumor cells in bone marrow (BM) and peripheral blood has become a major focus of translational cancer research. The presence of disseminated tumor cells in BM is a common phenomenon observed in 30–40% of primary breast cancer patients and independently predicts reduced clinical outcome. The detection of circulating tumor cells (CTCs) in blood might become a desired alternative to the invasive and painful BM biopsy. Recent clinical trials confirmed the feasibility of CTC detection as a robust and reproducible parameter for prognostication in both adjuvant and metastatic setting. The characterisation of CTCs might become an important biomarker for therapy monitoring and help to identify specific targets for novel therapeutic strategies (6).

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14) Galectin-3

Galectin (GAL)-3 is a b-galactoside binding lectin with roles in diverse processes including proliferation, apoptosis, inflammation and fibrosis. GAL-3 is expressed by various types of human cells that is commonly seen in cancer and pre-cancerous conditions, GAL-3 is an important regulator of a broad range of cancer cell activities including growth, transformation, apoptosis, angiogenesis, adhesion, invasion and metastasis (1).
GAL-3 is a novel serum tumor marker, and elevated levels correlate with cancer progression and metastasis (2-5).
The expression rate of GAL-3 in NSCLC tissue was 83.8% (52/62), which was significantly higher than that in normal lung tissue ($P<0.01$). GAL-3 expression was not correlated with sex ($\chi Ç=0.113$, $P>0.05$), age ($\chi Ç=0.220$, $P>0.05$), and histological type ($\chi Ç=0.012$, $P>0.05$), but was negatively correlated with pathological differentiation ($r=-0.292$, $P<0.05$) and positively correlated with the clinical stage ($r=0.336$, $P<0.05$) in NSCLC tissue. RT-PCR results showed that the expression level of GAL-3 in NSCLC tissue was significantly higher than in normal lung tissue. GAL-3 may be useful in lung cancer diagnosis and may increase diagnosis rate when combined with other lung cancer markers (6).
A novel marker with potential clinical importance in cancer progression as well as inflammation and fibrosis, has been extensively researched in multiple in vitro, in vivo, and epidemiological studies. Elevated levels have been shown to play a particularly significant role in promoting inflammation and fibrosis (7).
Reduces GAL-3 expression and disease severity in experimental acute kidney injury. MCP-treated mice demonstrated reduced GAL-3 in association with decreased renal fibrosis, macrophages, proinflammatory cytokine expression and apoptosis (8).
GAL-3 provided useful information and correlation with other inflammatory markers. Monitoring serum GAL-3 as a marker for both inflammatory processes and cancer progression to a higher probability of metastasis may have clinical relevance. (9)
Patients with thyroid carcinoma had significantly higher serum concentration of galectin-3 than those with benign thyroid lesions (papillary hyperplasia and thyroid adenoma) and normal subjects ($P<0.001$). In patients with papillary thyroid carcinoma, GAL-3 positivity in the tumor tissue was associated with a significantly higher serum GAL-3 level in comparison with the negative cases ($P<0.05$). (10)

Central to fibrogenesis and the scarring of organs is the activation of fibroblasts into matrix-secreting myofibroblasts. Galectin-3 expression is up-regulated in established human fibrotic liver disease and is temporally and spatially related to the induction and resolution of experimental hepatic fibrosis. Disruption of the Galectin-3 gene blocks myofibroblast activation and procollagen (I) expression in vitro and in vivo, markedly attenuating liver fibrosis. Galectin-3 is required for TGF-mediated myofibroblast activation and matrix production (11)

Serum levels of matrix metalloproteinase-9 (MMP-9) and its substrate galectin-3 were assessed in 50 Hepatocellular carcinoma (HCC) patients, 30 cirrhotic patients in addition to

10 healthy subjects as a control group using enzyme linked immune-sorbent assay (ELISA). This study found that HCC patients with metastatic spread had a significant elevation of both serum galectin-3 and MMP-9 levels (P = 0.028 and <0.0001, respectively). In addition, galectin-3 level significantly increased in HCC patients with poor prognosis suffering from portal vein invasion (P = 0.014).
In the present study, circulating level of galectin-3, MMP-9 increased significantly in HCC as compared to the control group (P = 0.044 and 0.04, respectively) (12)

Serum levels should <18

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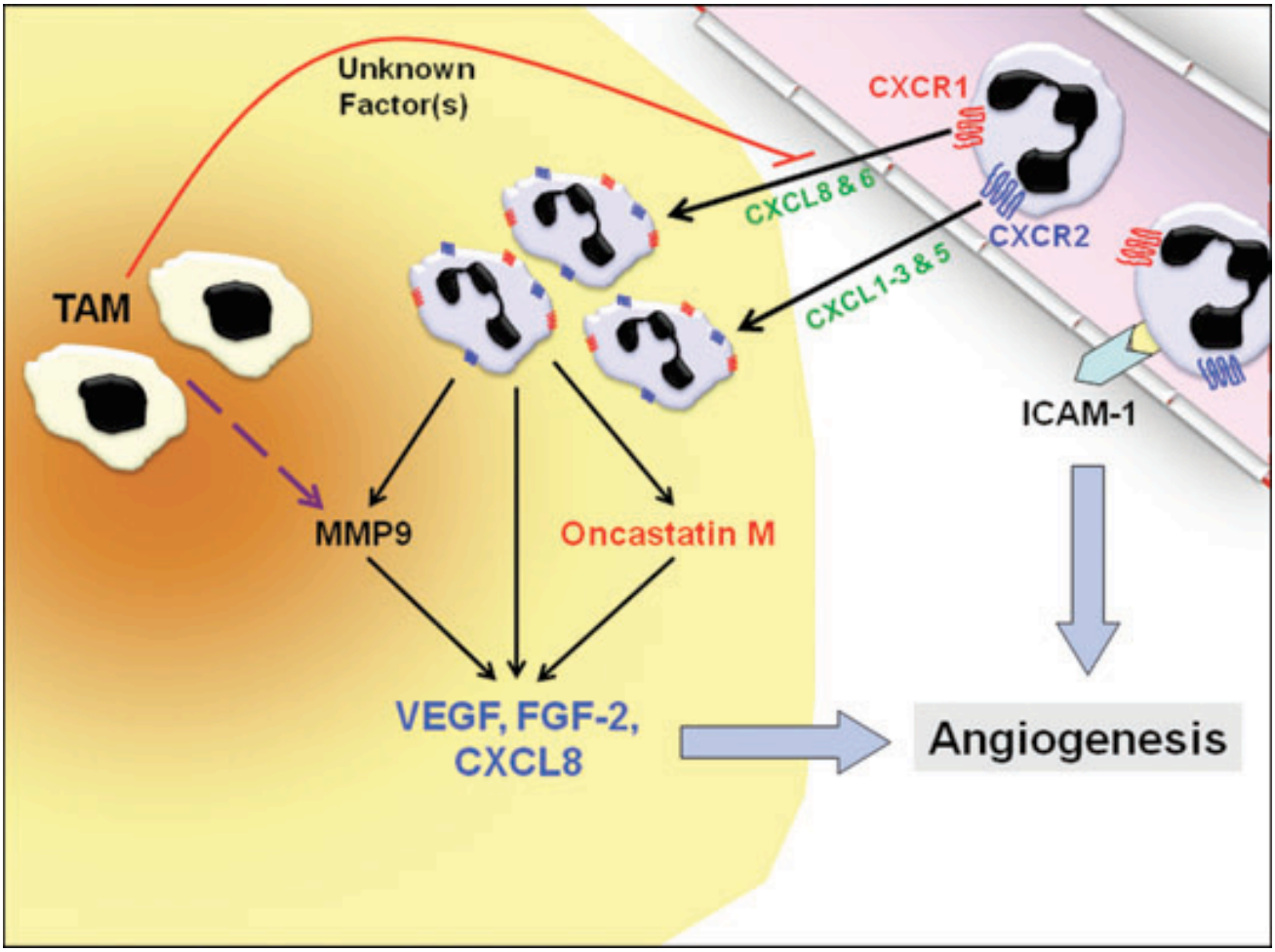
15) Neutrophil to lymphocyte ratio

- The neutrophil to lymphocyte ratio (N/L ratio) has been reported to be related to the prognosis of various types of cancer.

- In particular, a high N/L ratio has been be associated with poor outcome and suggest the need for botanical based immune enhancing therapy. (1)
- Several studies have demonstrated that raised preoperative N/L ratio is associated with poor prognosis in colorectal cancer (CRC). (2, 3, 4, 5)
- Preoperative N/L ratio, in combination with CA125, may represent a simple and cost-effective method of identifying ovarian cancers, and an elevated N/L ratio may predict an adverse outcome in ovarian cancer. (6)
- The level of preoperative N/L ratio and the change of preoperative/postoperative N/L ratio level tended to associate with PFS more than overall survival of epithelial ovarian cancer patient. (7,8)
- Combination of neutrophil lymphocyte ratio and platelet lymphocyte ratio is a useful predictor of postoperative survival in patients with esophageal squamous cell carcinoma. (9)
- N/L ratio and platelet lymphocyte ratio, are independent prognostic factor for OS in patients with advanced gastric cancer treated with chemotherapy. (10)
- Neutrophil and platelet-to-lymphocyte ratio are new predictors of dropout and recurrence after liver transplantation (LT) for hepatocellular cancer (HCC) - N/L ratio being a good predictor for the risk of dropout, while platelet lymphocyte ratio is a good predictor for the risk of post-LT recurrence. Use of these markers, which are all available before liver transplantation, may represent an additional tool to refine the selection criteria of HCC liver recipients. (11)

Neutrophils may be key mediators of tumor angiogenesis

- Neutrophils are recruited into the tumor and then elaborate on how these cells may induce tumor vascularization by the secretion of powerful pro-angiogenic factors. (12,13)
- In colon cancer patients increased numbers of neutrophils in these patients correlated significantly with poor prognosis. Increased numbers of neutrophils have also been observed in patients with myxofibrosarcoma, gastric carcinoma and melanoma. (14)



Neutrophil-induced tumor angiogenesis. Neutrophils are recruited into the tumor from the circulation via the production of chemokines for CXCR1 (CXCL8 & 6) and/or CXCR2 (CXCL1–3 & 5). Once inside the tumor, neutrophils can secrete factors such as oncostatin M, which stimulates tumor cells to increase their vascular endothelial growth factor (VEGF) production. Neutrophils also secrete tissue inhibitors of metalloproteinase (TIMP)-free matrix metalloproteinase (MMP)9 that can act in concert with MMP9 released by tumour-associated macrophages (TAMs) to liberate pro-angiogenic growth factors (VEGF & FGF-2) that are sequestered to the extracellular matrix (ECM). Cytokine-activated neutrophils also secrete VEGF and CXCL8 by de-granulation. These potent pro-angiogenic factors then act directly on the nearby vasculature to promote tumor angiogenesis.
In addition, the interaction of neutrophils with adhesion molecules (ICAM-1) on the endothelial cell surface may also stimulate angiogenesis. Interestingly, in an experimental tumor model, TAMs have been found to inhibit the infiltration of neutrophils by an as yet unidentified mechanism. (12,15)

High Neutrophil to Lymphocyte Ratio in Pancreatic Cancer Predicts Shorter Overall Survival

In advanced pancreatic cancer, a high neutrophil to lymphocyte ratio (NAL) predicts shorter overall survival. Both neutrophils and lymphocytes are known markers of inflammation, a predictor of worse outcome regardless of tumor type, according to David Goldstein MD, University of New South Wales, Sydney, Australia, and colleagues.

In the original study, 861 patients with metastatic pancreatic cancer were randomized to receive either nab-paclitaxel 125 mg/m2 plus gemcitabine 1,000 mg/m2 on days 1, 8, and 15 of each 28-day cycle, or, gemcitabine 1,000 mg/m2 weekly for 7 weeks followed by 1 week

of rest (cycle 1) and then days 1, 8, and 15 of each 28-day cycle (cycle ≥2). Findings showed a significant improvement in overall survival among patients who were treated with the combination therapy compared with those who received gemcitabine alone.

In the current updated analysis, the overall survival continued to show a benefit for the combination therapy: 8.7 months versus 6.6 months (hazard ratio [HR], 0.72; *P* = .001) A further look at the data identified other negative prognostic factors including the patient's age, the presence of liver metastases, low Karnofsky Performance Status, and having a NAL ratio of ≤5. NAL, a marker of systemic inflammation, at baseline, is also a very important negative prognostic factor," said Dr. Goldstein. When the NAL ratio was ≤5, overall survival was 9.1 months; when the NAL ratio was >5, overall survival was 5.0 months (HR, 1.839; *P*< .001).

Prior to this analysis, CA 19-9 had been accepted as a predictor of negative outcomes regardless of the tumor type. However, the researchers found that baseline CA-19-9 did not have an impact on outcome. Regardless of whether the patient's CA-19-9 was greater than or less than the median, the between-group difference in overall survival was not significant (8.1 vs 7.0 months, respectively).

"CA-19-9 ceased to be an important prognostic factor but was replaced by the NAL ratio, and this is a very potent factor," said Dr. Goldstein. "The hazard ratio was 0.57. It suggests a relationship biologically between the presence of inflammation and heightened tumor marker. It's a complex multi-factorial environment and it's not just the lymphocytes (16)."

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Neutrophil to lymphocyte ratio may be predict of mortality in all conditions

White blood cell (WBC) count is one of the useful inflammatory biomarkers in clinical practice. Although WBC is in normal range, subtypes of WBC like N/L ratio may predict cardiovascular mortality. N/L ratio is a readily measurable laboratory marker used to evaluate systemic inflammation. Because hypertension, diabetes mellitus, metabolic syndrome (1), left ventricular dysfunction, acute coronary syndromes, valvular heart disease, abnormal thyroid function tests, renal or hepatic dysfunction, known malignancy (2,3,7), local or systemic infection, previous history of infection (<3 months), inflammatory diseases, and any medication that related to inflammatory condition of patients, the measurement of N/L ratio can be potentially affected in all of above conditions. For these reasons, it would be better, if the authors had mentioned these factors.
Not only N/L ratio but also mean platelet volume, red cell distribution width (4), platelet distribution width, CRP, uric acid and gamma-glutamyl transferase (5) are easy markers to evaluate the prognosis of colon cancer patients (6). However, one should keep in mind that N/L ratio itself alone without other inflammatory markers may not give exact information to clinicians about the prognosis of colon cancer patients. So, from that point of view we think that it should be evaluated accompanied with other serum inflammatory markers (8,9).

Neutrophil-lymphocyte ratio as a predictor of outcomes for patients with hepatocellular carcinoma undergoing TAE combined with Sorafenib.

The aim of this study was to investigate the prognostic significance of blood NLR in patients with intermediate-advanced hepatocellular carcinoma (HCC) who received transcatheter arterial embolization (TAE) combined with Sorafenib. A total of 40 patients with intermediate-advanced HCC from January 1, 2010, through May 31, 2013, treated with concurrent TAE in combination with Sorafenib were admitted to this study in our hospital. Potential prognostic factors, including serum NLR, were analyzed. The pretreatment mean NLR was 3.0; 21 (52.5 %) patients with elevated high NLR (>3.0). The median survival of patients with a high NLR was 14 months (95 % CI 10.1-17.9 months) compared with 26 months (95 % CI 17.4-34.6 months) for patients with a low NLR; a significant difference was found in overall survival (P = 0 0.001). Barcelona Clinical Liver Cancer staging classification and NLR >3.0 were all predictors of poorer over survival. Multivariate analysis showed that high NLR was independent factors associated with worse survival. A high periprocedural NLR independently predicts poor survival in patients with unresectable HCC undergoing TAE combined with Sorafenib (10).

Neutrophil-lymphocyte ratio predicts overall and recurrence-free survival after liver transplantation for hepatocellular carcinoma.

AIM:
The goal of this study is to evaluate whether an elevated neutrophil-lymphocyte ratio (NLR) at the time of diagnosis predicts survival of patients with hepatocellular carcinoma (HCC) after liver transplantation (LT). We hypothesize that the NLR is predictive of overall survival (OS) and recurrence-free survival (RFS) in patients with HCC who undergo LT.

METHODS:
This is a retrospective analysis of adult patients undergoing LT for HCC between 2000 and 2008 at our institution. We define an elevated NLR as a ratio of 5 or greater.

RESULTS:
We included 160 patients who underwent LT for HCC in the time period, of whom 28 had an elevated NLR. Seventeen subjects experienced recurrent HCC during the study period. The cumulative survival among subjects with an elevated NLR was significantly lower than among subjects with a normal NLR. On univariate analysis, several factors (including an elevated NLR) predicted decreased OS and RFS. However, after multivariate analysis, only three factors (including elevated NLR) remained significant as predictors of OS. Additionally, multivariate analysis revealed that an elevated NLR was the only significant independent predictor of RFS.

CONCLUSION:
Preoperative NLR is a powerful independent predictor of OS and RFS in patients undergoing LT for HCC. Measurement of NLR could serve as a useful and easily obtained adjunct to the Model for End-Stage Liver Disease score and Milan criteria when evaluating this patient population and determining which patients will gain the most survival benefit from transplantation (11).

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16) The platelet contribution to cancer progression

- Complex interactions between tumor cells and circulating platelets play an important role in cancer growth and dissemination, and a growing body of evidence supports a role for physiologic platelet receptors and platelet agonists in cancer metastases and angiogenesis.
- Platelets contribute to the hypercoagulable state frequently observed in cancer patients, leading to an increased risk of venous thromboembolism (VTE). In previous studies a high platelet count was repeatedly found to be associated with an elevated risk of VTE and a worse prognosis in patients with cancer.
- Platelets provide a pro-coagulant surface facilitating amplification of cancer-related coagulation, and can be recruited to shroud tumor cells, thereby shielding them from immune responses, and facilitate cancer growth and dissemination.
- Platelets can support various steps of cancer development and progression by promoting tumor growth, angiogenesis and metastasis. Moreover, The role of platelets in malignancy involves:
    1. Tumor cell induced platelet aggregation can occur following tumor cell intravasation into the vasculature, thereby protecting or cloaking circulating tumor cells from physical clearance and immune surveillance,
    2. Platelets facilitate tumor cell arrest within the vasculature, endothelial cell retraction and subsequent tissue invasion,
    3. Platelets induce endothelial cell proliferation and new blood vessel formation, which are requisite for tumor associated angiogenesis and growth;
    4. Platelet-tumor and platelet stromal interactions in the tumor microenvironment depend, in part, on platelet activation and platelet protein release, which contribute to the inflammatory response. Additional platelet-related proteins and metabolites that facilitate proteolysis and tissue remodeling also enhance tumor growth and metastasis

(including bony metastases). (1,2)

- Thrombocytopenia experimentally induced by a variety of mechanisms has also been associated with a reduction in the number of metastases in tumor transplant models. (3,4)
- The combination of platelet count and neutrophil to lymphocyte ratio is a useful biomarker for predicting the postoperative survival of patients with colorectal cancer. (5)
- Elevated platelet count represents an independent adverse prognostic factor in patients with **metastatic breast cancer**. Thus metastatic breast cancer joins a range of cancers in which this easily measurable value can be used for clinical prognostication. (6)
- **Platelet Count Measured Prior to Cancer Development Is a Risk Factor for Future Symptomatic Venous Thromboembolism: The Tromsø Study -** Elevated platelet count is associated with risk of venous thromboembolism in cancer patients initiating chemotherapy. It is not known whether this risk by platelet count is causal or merely reflects the malignant disease. We investigated whether pre-cancer platelet count alone or together with high leukocyte count was associated with risk of venous thromboembolism in subjects who did and did not develop cancer during follow-up in a population-based cohort study. Platelet count and other baseline characteristics were measured in 25160 initially cancer-free subjects who participated in the Tromsø Study in 1994-1995. Incident cancer and symptomatic venous thromboembolism events were registered up to December 31st, 2009. Multivariable Cox regression models were used to calculate hazard ratio for venous thromboembolism across categories of platelet count (<40th, 40-80th, and >80th percentile) with 95% confidence interval. During follow-up, 2082 subjects were diagnosed with cancer. Platelet count was measured on average 8.3 years before the cancer diagnosis. There were 129 venous thromboembolism events in the cancer cohort (13.5 per 1000 person-years) and 377 in the non-cancer cohort (1.2 per 1000 person-years). In cancer patients, pre-cancer platelet count above the 80th percentile (≥295×109/L) was associated with a 2-fold higher risk of venous thromboembolism (Hazard ratio: 1.98, 95% confidence interval 1.21-3.23) compared to platelet count below the 40th percentile (<235×109/L). Concomitant high platelet and leukocyte counts showed a synergistic effect on the VTE risk. In cancer-free subjects, no association was found. In conclusion, pre-cancer platelet count was associated with risk of symptomatic venous thromboembolism in cancer patients, but not in cancer-free subjects. Our findings suggest that platelet count and platelet-leukocyte interactions may play a role in the pathogenesis of cancer-related venous thromboembolism (7).

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17) Markers of Bone Metastasis: Bone-specific Alkaline Phosphatate (BSAP), N-Telopeptide (NTX), Calcium

The use of bone turnover markers in oncology includes monitoring of anticancer treatment in patients with malignant disease metastatic to the bones (therapeutic monitoring), predicting the risk of bone relapse in patients with a first diagnosis of potentially curative, early-stage malignant tumors (prognostic use), and making an early diagnosis of (microscopic) malignant bone disease in patients with a known malignant tumor to start early bone-targeted treatment and avoid skeletal-related events (diagnostic use) (1).

- **Bone-specific Alkaline Phosphatate (BSAP)**
  Plasma BSAP level accurately reflects osteoblastic activity and can be useful in metastatic o steoblastic-type breast cancer in the bone, whereas NTx is more useful in osteolytic-type b reast cancer in the bone (2).

- **N-telopeptides of Type I collagen (NTX)**
  Measurement of urinary NTX was valid diagnostic method for assessing bone metastasis (3). High NTX is the most accurate marker associated with elevated risks of both first Skeletal-related events (SRE) and overall survival (4). Also early normalization of elevated baseline NTX while receiving zoledronic acid is associated with longer event-free and overall survival times compared with persistently elevated NTX (5).
  In lung cancer urinary NTX and serum BALP have a high value in the diagnosis, therapeutic effect monitoring and SRE prediction of NSC Lung Cancer with bone metastases (6). In breast, prostate and lung cancer normalized NTX within 3 months of zoledronic acid treatment, versus persistently elevated NTX, was associated with reduced risks of skeletal complications and overall survival (7).

  NTX and BSAP are widely used bone metabolism markers, and offer reliable surrogate markers to detect bone metastatic spread and to predict prognosis for prostate cancer patients with bone metastases (8).

- **Serum Calcium**
  Elevated serum calcium (especially with elevated vitamin D diH 125), is a marker for hypercalcemia of malignancy is a common paraneoplastic syndrome and a frequent complication of advanced breast and lung cancer, and multiple myeloma (9).

- **Osteocalcin**, a marker in diseases with elevated bone metabolism
  Osteocalcin is synthesized by osteoblasts and its concentration in serum is increased when bone metabolism is raised. Radioimmunoassay of serum from 88 healthy adults gave a mean osteocalcin value for the whole group of 4.11 +/- 1.43 ng/ml. The level rose with age. In seven patients with primary hyperparathyroidism the mean value was markedly raised to 19.37 +/- 9.2 ng/ml, in 23 with metastasizing carcinoma of the breast it was elevated to 6.57 +/- 2.98 ng/ml. (10)

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18) Cortisol (Blood/saliva) & DHEA Sulfate

Monitoring cortisol would be beneficial for evaluating the quality of life of breast cancer patients on chemotherapy.

Short-term memory (STM) decline in breast cancer patients resulting from chemotherapy was evaluated by means of blood biomarkers, a questionnaire, and a computerized STM test This study was conducted from January 2013 to June 2013, recruiting 90 subjects: 30 breast cancer patients beginning the 3rd of 4th cycles of docetaxel and cyclophosphamide chemotherapy, 30 recovered patients (who completed 4 cycles of docetaxel for a minimum of 6 months), and 30 healthy subjects (disease-free females). The levels of hemoglobin, red and white blood cells, and cortisol in serum, and a computerized STM test were analyzed to estimate the effects of chemotherapy on STM. Statistically significant differences were observed for the blood parameters (hemoglobin, red and white blood cells, and cortisol levels) between healthy and on-treatment subjects. depleted levels of hemoglobin, red and white blood cells as a result of chemotherapy, and elevated levels of stress correlated with poor performances in the computerized STM test. A higher cortisol level might be an important precursor of STM deterioration (1).

24-Hour Cortisol Test

The satisfactory precision of the analysis and the simple non-invasive sampling procedure suggest that saliva may be used for cortisol measurements in situations where blood sampling is difficult to perform. (2) Perceived stress correlated with increases of cortisol levels during the first hour after awakening and this subgroup also showed the lowest self-esteem, the highest external locus of control, and the highest number of somatic complaints. (3) Abnormal circadian rhythms have been observed in patients with cancer. (4) Cancer, stress, and elevated cortisol levels are linked. In a 1996 case-controlled study, scientists examined hormone levels of the hypothalamic-pituitary-adrenal system in women with both early-stage and metastatic breast cancer. (5) Both groups had statistically higher levels of cortisol

compared to women without breast cancer. Cortisol slope predicted subsequent survival up to 7 years later. Earlier mortality occurred among patients with relatively "flat" rhythms, indicating a lack of normal diurnal variation. (6)

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19) Melatonin (saliva)

The assay of saliva is an increasing area of research with implications for basic and clinical purposes. Recently, the use of saliva has provided a substantial addition to the diagnostic armamentarium as an investigative tool for disease processes and disorders. (1) Saliva specimens provide an economical and practical method for melatonin assessment, however, in older adults, issues such as hyposalivation and low melatonin levels limit the feasibility and validity, respectively, of saliva melatonin. (2)
In 1985 Christian Bartsch and colleagues at the University of Tubingen discovered that prostate cancer patients had an abnormal melatonin secretion pattern and concluded that melatonin secretion may be related to the development and growth of prostate cancer. (3) Chinese researchers, treating a patient with terminal, metastatic prostate cancer and rising PSA levels with 5 mg a day of melatonin (given at 8 PM), found that this therapy stabilized his disease for 6 weeks as indicated by stable PSA levels. (4) Women who predominantly work at night have a 50% increased risk of developing breast cancer. The researchers conclude that exposure to light during the night suppresses melatonin production and hence increases the risk of cancer. (5) There is a significant correlation between melatonin levels and the presence of endometrial cancer. The mean plasma melatonin value was 6.1 pg/ml in the cancer-positive group and 33.2 pg/ml in the cancer-negative control group resulting in a 6-fold difference between the two groups. (6)
Inappropriately low melatonin level is a risk factor for prostate (and breast) cancer. There is evidence that a low level can be avoided by sleeping in a completely dark room at night and by ensuring that the ambient EMF level is low. Although the officially sanctioned safe continuous exposure level in the United States is 1000 mG (7), there is evidence that an exposure level of only 5 mG significantly reduces melatonin levels. (8)

Women with polycystic ovary syndrome (PCOS) were found to have a significantly higher melatonin level at 08:00 a.m. and smaller mean night-day difference in the concentrations of melatonin in comparison with those of healthy women (natural log (Ln) night-day difference 0.60 +/- 0.10 pg/ml versus 1.15 +/- 0.14, $p < 0.002$). (9)

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