WJCO World Journal of Clinical Oncology

Online Submissions: http://www.wjgnet.com/2218-4333office
wjco@wjgnet.com
doi:10.5306/wjco.v2.i6.245

World J Clin Oncol 2011 June 10; 2(6): 245-261
ISSN 2218-4333 (online)
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REVIEW

Potential of soluble CD26 as a serum marker for colorectal cancer detection

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Supported by (in part) A grant from Xunta de Galicia (10 PXIB 310 215 PR) and FEDER founding
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Received: January 21, 2011 Revised: March 28, 2011
Accepted: April 5, 2011
Published online: June 10, 2011

Abstract

Colorectal cancer is characterized by a low survival rate even though the basis for colon cancer development, which involves the evolution of adenomas to carcinoma, is known. Moreover, the mortality rates continue to rise in economically transitioning countries although there is the opportunity to intervene in the natural history of the adenoma–cancer sequence through risk factors, screening, and treatment. Screening in particular accounted for most of the decline in colorectal cancer mortality achieved in the USA during the period 1975-2000. Patients show a better prognosis when the neoplasm is diagnosed early. Among the variety of screening strategies, the methods range from invasive and costly procedures such as colonoscopy to more low-cost and non-invasive tests such as the fecal occult blood test (guaiac and immunochemical). As a non-invasive biological serum marker would be of great benefit because of the performance of the test, several biomarkers, including cytologic assays, DNA and mRNA, and soluble proteins, have been studied. We found that the soluble CD26 (sCD26) concentration is diminished in serum of colorectal cancer patients compared to healthy donors, suggesting the potential utility of a sCD26 immunochemical detection test for early diagnosis. sCD26 originates from plasma membrane CD26 lacking its transmembrane and cytoplasmic domains. Some 90%–95% of sCD26 has been associated with serum dipeptidyl peptidase IV (DPP-IV) activity. DPP-IV, assigned to the CD26 cluster, is a pleiotropic enzyme expressed mainly on epithelial cells and lymphocytes. Our studies intended to validate this test for population screening to detect colorectal cancer and advanced adenomas are reviewed here.

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Key words: Antibody arrays; Bioinformatics; Biomarkers; cancer; Clustering; Cytometric beads; Diagnosis; Dipeptidyl peptidase Ⅳ; Enzyme linked immunosorbent assay; Prognosis; Soluble CD26; Screening; Serum

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Cordero OJ, Imbernon M, De Chiara L, Martinez-Zorzano VS, Ayude D, Paez de la Cadena M, Rodriguez-Berrocal FJ. Potential of soluble CD26 as a serum marker for colorectal cancer detection. *World J Clin Oncol* 2011; 2(6): 245-261 Available

from: URL: http://www.wjgnet.com/2218-4333/full/v2/i6/245.htm DOI: http://dx.doi.org/10.5306/wjco.v2.i6.245

EARLY DIAGNOSIS AND POPULATION SCREENING FOR COLORECTAL CANCER

Colorectal cancer (CRC) clearly meets all the required conditions for the adoption of a screening policy.

First, because it is an important issue for public health since it is one of the most common cancers (ranking third both in men and women) worldwide and because it is characterized by a low survival rate due to diagnosis in advanced stages, which leads to high mortality rates. For example, in the United States the American Cancer Society estimated that in 2010 there were 142 570 new cases and 51 370 related deaths from colon cancer[1] and more than 1 million new cases and about 530 000 deaths worldwide[2]. Moreover, globally, while in all developed countries CRC rates have stabilized or are declining[3], CRC incidence in economically transitioning countries continues to rise both in its incidence and in mortality because of increased exposure to risk factors[2, 3].

The second condition is that the basis of colon cancer development is well known and involves the evolution of adenomas to carcinoma[4,5], therefore, individuals with a history of adenomas have a higher risk of cancer[6] and removal of polyps results in a reduction in colon cancer incidence[7]. However, we have the opportunity to intervene in the natural history of the adenoma–cancer sequence[8].

Third, there are precise and feasible diagnostic methods that allow detection of the disease in early stages (non-metastatic tumors), which could be surgically cured by removal (reduction in the mortality rate of CRC), as well as the identification and removal of polyps (reduction in the incidence rate of CRC)[9-11]. Moreover, treatment is more effective and patients show a better prognosis when the neoplasm is diagnosed early[12].

Interestingly, it has recently been reported that screening accounted for 53% of the decline in CRC mortality observed between 1975-2000 in the USA (26% less mortality); the other two facts being changes in risk factors (35%) and treatment regimes (12%)[8]. Moreover, the decline in CRC mortality in the USA can be enhanced if current trends, including screening, against cancer are accelerated; for example, only approximately 50% of its population older than 50 years have been screened[8]. Needless to say that in most countries, including many developed countries, no screening strategy has been proposed.

There are a great variety of screening strategies available for the average risk population, that is, individuals of, or over, 50 years with no other known risk factors for the development of CRC. These methods range from invasive and costly procedures such as flexible sigmoidoscopy, double contrast barium enema, and colonoscopy to more low-cost and non-invasive tests such as the fecal occult blood test (FOBT). All these methods have advantages and disadvantages regarding their sensitivity, specificity, risk, availability and cost but they have been shown to decrease CRC incidence and mortality[8, 13-15].

Colonoscopy is the gold standard[15] and multiple studies have provided indirect evidence regarding the higher benefits of colonoscopy compared with other methods[16]. However, the costs and risk of complications, besides discomfort, have made this and other invasive tests such as flexible sigmoidoscopy[17], poorly accepted for screening in an asymptomatic population[18- 23].

The benefit of CRC screening using a non-invasive test for blood in stool (Hemoccult) was established in 1993[13]. Subsequently, this result was corroborated in two other randomized controlled trials, leading to recommendations in many countries for CRC screening[24, 25]. The FOBT is the simplest and least expensive non-invasive approach to CRC screening available, however, it has several disadvantages. The most common method is the non-rehydrated guaiac FOBT[26], based on the detection of peroxidase activity in the stool sample. Consequently, reagents also bind to nonhuman hemoglobin-like substances in feces, such as animal myoglobin and plant peroxidases. As the presence of these substances in the colon and rectum are related to diet, important dietary restrictions are required to minimize false negative results[13, 27]. Notwithstanding, its sensitivity and specificity are 30%-40% and 96%-98%, respectively[28], with lower percentages for the detection of adenomas[29, 30].

In the United States, the current recommendations include a number of screening tests in addition to Hemoccult. Immunochemical tests (iFOBT), which have not been evaluated in a randomized controlled trial, have performed similarly or even better in some studies, with generally higher compliance rates compared to Hemoccult or other guaiac-based tests[27, 31-34], involving no dietary restriction, and resulting in fewer false positives[35]. The use of an immunochemical test in patients scheduled for colonoscopy[36] showed the advantages of a quantitative test to determine the cutoff for positivity to adjust the screening program according to the resources available. Moreover, this test can be automated and two instead of three samples can be used for quantification[37].

In Japan, more than 6 million people have been screened with immunochemical tests, with a positivity rate of 7.1%[35, 38-40]. With 60% of positive tests complying with the diagnostic protocol, the CRC detection rate was 1.6 per 1000. More than 70% of the cancers were classified as Duke's A or Duke's B, suggesting that the program worked well in detecting early stage cancer; CRC mortality and incidence were reduced by 72% and 59%, respectively[41]. Somewhat puzzling is the fact that guaiac is more sensitive than immunochemical for advanced adenomas (41.3% *vs* 29.5%)[42], this may be because peroxidase sensitivity of the guaiac test detects lower levels of bleeding as some authors speculate. However, other explanations must also be considered.

This last study does illustrate the utility of comparing

different tests rather than conducting long-term and expensive randomized controlled trials to evaluate each new FOBT. There are considerable data on Hemoccult, therefore comparing performance, outcome, compliance, and cost with new blood or fecal occult tests, as was done in this study[42], should be enough for the acceptance of new tests.

As blood could be present in the stool for other reasons, such as hemorrhoidal bleeding, iFOBT was also tested in combination with protein stool markers like hemoglobin-haptoglobin, calprotectin, carcinoembryogenic antigen, and the novel fecal markers S100A12 and tissue inhibitor of metalloproteinase-1 (TIMP-1), the latter allowing the detection of CRC at significantly higher rates than can be obtained with iFOBT alone[43]. Genetic markers are also promising tools, such as the DNA-based stool test *PreGen-Plus* from EXACT Sciences, which shows a sensitivity of 51%-91% for CRC, with an average of 65%, and specificity between 93%-98%[30,44].

However, non-invasive biological serum markers would be of great benefit for screening, because blood-based diagnostics can additionally classify tumors into distinct molecular subtypes and monitor disease relapse and response to treatment. Increasingly, biomarker strategies are becoming critical to identify a specific patient subpopulation that is likely to respond to a new therapeutic agent. The improved understanding of the underlying molecular features of common cancers and the availability of a multitude of recently developed technologies to interrogate the genome, transcriptome, proteome and metabolome of tumors and biological fluids have made it possible to develop clinically applicable and cost-effective tests for many common cancers[45,46].

SERUM BIOMARKERS IN CRC SCREENING

Other advantages over stool testing are: sampling may be more convenient and acceptable for the patient, there is no microflora which could degrade the biomarker or hamper analysis, and sample processing may be easier. In addition, as it will be commented later, information on the very early pathways of carcinogenesis, such as immune system cross-talk, can only be found in serum.

A meta-analysis evaluating blood markers for early detection of CRC reported in 2007 summarizing the performance characteristics of various approaches[47] found that seventy different markers fulfilled the inclusion criteria with an overall sensitivity that ranged from 18% to 65%. The markers included cytologic assays, DNA and mRNA markers, and soluble proteins.

Three studies investigated cytologic assays, an inhibition of *in vitro* leukocyte adherence by incubation with tumor antigens, and the detection of circulating tumor cells by a membrane array[48-50]. Sensitivity was above 70% for early stages and specificity ranged from 94% to 98%, however, the number of cases by tumor stage was very small. Notwithstanding, cellular mechanical properties have recently received increasing attention as a potential biophysical marker for cancer cells[51].

Four studies[52-55] with DNA markers for the early detection of CRC were reported in that review[47]. Free DNA, as well as mRNA, was isolated from circulating cells. Blood samples were analyzed for both genetic and epigenetic alterations of genes involved in the adenoma-carcinoma sequence, such as K-ras, tumor suppressor protein p53, APC (adenomatous polyposis of the colon), hMLH1 (human MutL homologue 1) or HLTF (helicase-like transcription factor). Sensitivity reported for this group of markers was about 60% and lower, whereas specificity ranged from 73% to 100%. The potential of detecting adenomas was investigated only for mutations in the *K-ras* gene in one study which showed a sensitivity of 35% for adenomas[54]. A recent review evaluated four commercialized biomarker tests based on that information (K-ras and B-raf mutation analyses, mismatch repair protein testing, and the Oncotype DX Colon Cancer Assay) for inclusion in the NCCN Guidelines Panel for Colon Cancer. In two cases, the available evidence was inconsistent to be included in the specific NCCN Guidelines[56].

Novel data on genetic and epigenetic mechanisms of CRC and how these alterations relate to emerging biomarkers for early detection, risk stratification, prognosis and prediction of treatment responses, are reviewed in[57-60]. Potential markers waiting to undergo clinical validation for response to therapies are hypermethylation of *septin-9* and *DPYD* (dihydropyrimidine dehydrogenase) genes.

Many relevant studies[47] applied reverse transcription-PCR to detect mRNA expressed in circulating tumor cells. Blood samples were analyzed for mRNA molecules coding for CEA, cytokeratins (CK) 8, 9, and 20, human telomerase reverse transcriptase (hTERT), guanylyl cyclase C (GCC), carcinoembryonic gene member 2 (CGM2), melanoma-associated antigen family A (uMAGE-A), tumor-associated antigen L6, mucins (MUC) 1 and 2, protease M (ProtM), and thymidylate synthase. The most promising performance characteristics in this group of markers were reported for GCC mRNA[61], showing above 80% sensitivity for early stages.

Recent research has shed light on the biological importance of microRNAs(miRNAs). Their association with formation, angiogenesis, metastasis, and chemo-therapy resistance of tumors has become one of the core issues in epigenetics of cancer, including CRC. miRNAs serve as micromanagers, negatively regulating gene expression. The potential utility of miRNAs in the preclinical stage has been explored, since manipulation of miRNAs may offer an alternative therapy for chemo- and radio-resistant CRCs[62-64].

The discovery that aberrantly expressed miRNAs vary among different tumor types and some of them are secreted in highly stable, cell-free form into blood[65] led to the hypothesis that circulating (and fecal) miRNAs might potentially serve as non-invasive markers for early diagnosis

of CRC[63]. For example, 69 miRNAs were detected in CRC but not in control group sera; of these, 12 were not found in the serum of lung cancer patients[64]. Circulating miRNAs are packed in complexes, either called exosomes or microvesicles, and emerging evidence has indicated that such external miRNAs are involved in cell-to-cell signal transduction and genetic information exchange[64,66]. Two miRNAs significantly elevated in plasma and CRC tissues, but reduced in postoperative samples when compared with preoperative samples are miR-17-3p and miR-92a, both belonging to the miR-17-92 cluster. At a cut-off value of 3.6 for miR-17-3p (relative expression in comparison with RNU6B), the sensitivity was 64% and the specificity was 70%; at a cut-off value of 240 for miR-92a, the sensitivity was 89% and the specificity was 70%. In addition, miR-92a can distinguish CRC from other gastrointestinal cancers and inflammatory bowel diseases as well as advanced adenoma from normal controls, with a sensitivity of 64.9% and a specificity of 81.4%, whereas its expression levels were not correlated with tumor-node-metastasis (TNM) stages[66,67].

Fifty two protein markers in the meta-analysis of 2007[47] were analyzed by common standard procedures, still more easy-to-use and quicker than the nucleic acid methods, like ELISA, RIA, or activity assays, or by chromatographic and mass spectrometric (MS) assays based on surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) MS, and matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS. This group of markers can be further subdivided into carbohydrate antigens, carcinoembryonic antigens, other antigens, antibodies, cytokines, and other proteins. Sometimes, different markers were analyzed in parallel. For example, combinations including carbohydrate antigens and carcinoembryonic antigens were very common.

Carcinoembryonic antigen (CEA) was the first blood marker proposed in connection with CRC[47, 68]. Although overall sensitivity ranged between 43% and 69%, there was a clear increase in sensitivity by tumor stage, ranging from 8% for Duke's A up to 89% for Duke's D. Specificity was above 90% in nineteen studies.

Carbohydrate antigens (defined by monoclonal antibodies against colon carcinoma cell lines) include CA 19-9, CA 195, CA M26, CA M29, CA 50, CA 72-4, CA M43 and CA 242. Many studies evaluated CA 19-9, with an overall sensitivity from 18% to 65%, and specificity above 90% in most studies. Sensitivities greater than 50% were only observed for nonlocalized disease. For other carbohydrate antigens, the observed sensitivity, its stage dependency, and specificity were comparable.

Early approaches for other antigens investigated sialylated Lewis X antigen (sLeX) and CO 29.11, another sialylated Lewis antigen. sLeX was originally found on tumor tissues by immunohistochemistry and CO 29.11 is expressed and shed by carcinoma cells of colon and other cancer types[69-77]. Later studies investigated the potential of PSA, PA 8-15 (another tumor-associated antigen that was originally observed in a pancreatic cancer cell line), small intestinal mucin antigen (SIMA), and urokinase-type plasminogen activator (u-PA). For the latter, a sensitivity of 76% (82% for non-mestatasized disease) and a specificity of 96% have been reported[69, 70].

Among various circulating autoantibodies against antigens such as DEADbox protein 48 (DDX-48), p53, sFasL (the death receptor ligand of CD95), or NCC-ST 439 (a tumor-related carbohydrate)[78-83], sensitivities for the detection of CRC hardly reached 30%, although specificity was 100% in all studies.

In studies[84-89] evaluating cytokine markers such as vascular endothelial growth factor (VEGF), insulin-like growth factor II (IGF-Ⅱ), IGF-binding proteins (IGFBP-2), stem-cell factor (SCF), and interleukin-3 (IL-3) which can reflect several immune system-related pathways of carcinogenesis[90], if specificity was high (between 90% and 100%), sensitivity was low (37% for VEGF in TNM Ⅰ stage patients), or *vice versa*.

Among the other proteins, subgroup examples are the α-defensins[91], the nicotinamide N-methyltransferase[92], the α-L-fucosidase[93] and the tumor M2-pyruvate kinase (M2-PK), an isoform of the glycolytic enzyme pyruvate kinase[94]. Recent works also studied other potential markers in relation to polyp characteristics: for serum sulfatase activity, differences regarding the number of adenomas (single or multiple) were significant[95]; serum leptin, adiponectin and resistin also differed between controls and patients with adenomas or CRC, although there was no relationship with dysplasia, histopathology or polyp localization[96].

One of the signatures of a cancer cell is the change in the nuclear structure and architecture, and alterations in the composition of nuclear structural proteins are associated with various types of cancer such as breast, prostate, bladder, lung and ovarian, as well as squamous cell carcinoma of the neck[97, 98]. Nuclear proteins, colon cancer–specific antigen (CCSA)-2, CCSA-3, and CCSA-4 were recently identified as serum biomarkers that are specific for colon cancer[99,100].

As a summary of these and more recent studies, the more promising results, for both sensitivity and specificity, were observed with u-PA (76% and 96% respectively[61], M2-PK[101] (69% and 90%), TPA-M (70% and 96%), CP (cancer procoagulant; 86% and 82%)[102], sCD26 (soluble cluster of differentiation 26) (90% and 90%[103], fibrin degradation (DR-70) (80% and 93%)[104], prolactin (77% and 98%)[105], laminin (89% and 88%)[106], BSP (bone sialoprotein; 88% and 100%, although similar results were found in breast and prostate cancers)[107] and CCSA-2 (78% and 97%), but sCD26 has been the most studied, as commented in later subheadings[108-111].

SOLUBLE CD26

Dipeptidyl peptidase Ⅳ (DPP-Ⅳ), assigned to the CD26 cluster, is a multifunctional or pleiotropic protein expressed particularly on epithelial cells and lymphocytes. CD26/DPP-IV has been consistently associated with

cancer since it was known as ADCP, or the ADA-2 / Large isoform[112,113]. Many reviews have discussed the non-enzymatic role of CD26/DPP-IV as an extracellular anchorage for ADA in cancer and the potential usefulness of this protein in therapeutics and diagnostics[114-119]. The ADA-CD26 complexes may participate in cell-to-cell contacts[120-122] or, more probably in this context, through the catalysis of adenosine to inosine[121,123,124]. Proliferating cells accumulate high extracellular concentrations of adenosine, a purine nucleoside found within the interstitial fluid of solid tumors, which may be toxic or influence the proliferative potential of a cell, depending on the relative expression and type of adenosine receptor (AR). Therefore, the different levels of the cell-surface CD26-ADA complex and relative expression of ARs on a tumor cell may lead to the generation of tumor subclones, as well as its participation in the well-known adenosine inhibition of cell-mediated immune responses to tumor cells[115,116,119,124-127]. Other pro-oncogenic activities may be related to the recently described CD26-ADA-plasminogen ternary complex. Binding of plasminogen to cell-surface receptors promotes its conversion to plasmin, which is required for proteolysis of the ECM in several physiological and pathological processes, including cell migration, tumor cell invasion and metastasis[127].

CD26, also present at the invadopodia, together with other ectoproteases and metalloproteases (MMPs)[128-130], can participate in malignant transformation and cancer progression through its ability to bind collagen and fibronectin[81,116,117,128,129,131,132]. MMPs and FAPα(a CD26 homologous protein expressed in tumor cells) digestion of ECM components will allow passage of the malignant cells through basement membranes and stromal barriers. This pro-oncogenic behaviour is thus consistent with the non-enzymatic interactions with cell-surface ADA and plasminogen mentioned above, and the formation of FAP-CD26 heterodimers[129, 133].

However, there is a fundamental difference between CD26 and the other proteases involved in cancer development and progression as executors of ECM degradation: CD26 is constitutively expressed in the tissues mentioned at the beginning of this heading, and its enzymatic activity regulates the biological activity of regulatory peptides, such as incretins secreted by the enteroendocrine system (DPP-IV has therefore become a novel therapeutic target for inhibitors that extend the endogenously-produced insulin half-life in diabetics [114,115,134-141], and similarly the half-life of growth factors and chemokines[142]).

In addition, glypican-3 has recently been reported as the first natural inhibitor of CD26/DPP-IV enzymatic activity, in *in vitro* experiments[143]. Glypicans are basically absent in adult tissues, but up-regulated in many tumor tissues[144]. If glypican-3-dependent local DPP-IV inhibition can be confirmed in a physiological context, this indicates a natural protective role for the enzyme that should be blocked in the tumorigenic process.

This anti-oncogenic role, first contrasted in 1999 by Houghton's group[145-148], together with many data -differences in the cellular staining pattern with respect to the normal tissue, significant intratumor heterogeneity and changes in CD26 expression linked to the transition of tumor stages- already reviewed[115], indicate a quite complex situation in the physiological microenvironment of cancer niches. The possibility that the tumorigenic process may manipulate the functions of CD26/DPP-IV, for example evading the immune system by modifying local chemokine gradients (and therefore, immune cell homing), and by modulating cytokines and angiogenic or immunosuppressive factors[90,149-153] deserve to be studied in more detail[142].

In this context, the role of serum DPP-IV activity, first discovered in 1968 by Nagatsu's group in Japan[154], is not known. Within normal plasma/serum, some 90%–95% of DPP-IV activity has been associated with a relatively high concentration of serum (or soluble, in contrast to transmembrane) CD26 (sCD26) in human serum (570 μg L-1)[115,118,155-157]. Since sCD26 is heavily glycosylated, its molecular weight is similar to that of transmembrane CD26[156,158] although it lacks transmembrane and cytoplasmic domains (the sequence starting at the 39th position)[156].

There is no direct correlation between serum CD26 protein concentrations and serum enzymatic activity assays, for three reasons: (1) There are some circulating proteins other than CD26 with DPP-IV activity (DPP-Ⅱ, FAPα,...); (2) sialylation (a type of glycosylation) of sCD26 [114,118,135-137,158] is strongly enhanced in elderly individuals[159], and certain type of hypersialylation can inhibit DPP-IV activity[160], consistent with the fact that serum/plasma DPP-IV enzymatic activity tends to decrease with age[118]; (3) it has recently been suggested that the serum protein attractin, which enhances the enzymatic activity of tolloid proteases[161,162], may regulate the DPP-IV activity of CD26/sCD26 in the same way [115]. Serum attractin is actually frequently co-purified with sCD26[163-166].

Iwaki-Egawa *et al*[156,167,168] suggested that sCD26 must be shed from any plasma membrane on CD26 expressing cells that are in contact with blood, by proteolytic cleavage. The fact that only one CD26 mRNA form is usually reported[169-171], and that it is transported from its site of synthesis in the rough endoplasmic reticulum to the microvillar membrane of enterocytes, and in some cell lines in a membrane-bound state[135,136,172-174], also suggest that it is not secreted. It must be pointed out that the shedding of most integral membrane proteins is often regulated by a PKC-dependent mechanism[175-177].

However, CD26 has been found to be soluble in the lumen of secretory granules, undergoing exocytosis to the interstitial space of endocrine pancreatic A cells, where sCD26 may act on secretory products of neighbouring islet cells[178,179]. Autolysis of the protein by the acidic pH conditions inside the granules has been observed *in vitro*[179,180]. In addition, another possibility related to the intracellular sorting is the secretion of soluble proteins through MMP-dependent shedding from exosomes. Exosomes are small membrane vesicles derived from

intracellular multivesicular bodies that can undergo constitutive and regulated secretion from cells upon fusion with the PM[181-183]. Exosomes with CD26/DPP-IV have been found in human saliva, released at the basolateral surface of enterocytes, and in ram epididymal fluid[184-186].

In addition, the origin of sCD26 is also unknown. The hepatobiliary system was the first to be suggested[187]. Liver epithelium is often cited as the most likely potential source[113, 116, 137, 188-192] and at least in some conditions, sCD26 originates from the brush border of hepatocytes[190]. However, CD26 is predominantly located in the bile canaliculi[190, 193, 194], and a recent study found that in chronic hepatitis C and other liver viral infections, DPP-IV activity levels were not correlated with several markers of bile duct injury or hepatocyte injury[195]. These authors suggested that the increased activity in these diseases may originate directly from its shedding from the peripheral blood T cells involved in the control of viral infections or, indirectly, by stimulating other cells such as hepatic stellate cells. The involvement of T cells had already been suggested in studies of liver regeneration[137,196]. In fact, Kasahara *et al*[197] suggested a possible origin of sCD26 from the immune system, although they also identified serum isoforms from liver, spleen or kidney. Kidney, an obvious potential source because it contains large amounts of CD26, was rejected early on[156] because anephric individuals have normal amounts of sCD26, and because sCD26 contains approximately twice as much sialic acid as kidney CD26. However, several data suggest that serum CD26 is at least partly shed from T cells[142, 158, 161, 194, 198-204], although these data do not preclude the possibility of sCD26 also being shed from the endothelium of venules or the capillary bed of several organs such as lung, myocardium and striated muscles, spleen and pancreas[134, 194, 199, 205-210]. Moreover, this fraction of serum CD26 which originated from immune system cells can be regulated[158, 211-213] and causes an imbalance among specific sCD26 isoforms in the serum of patients.

As it is not known to which CD26 functions regulation of this proteolytic or secretory process is related, the physiological role of soluble CD26 in biological fluids with respect to the transmembrane CD26 can only be hypothesized. Current data support three potential biological functions, which may be partly responsible for the different roles of CD26 in various clinical settings. (1) Involvement in the activation–deactivation of some chemokines, and therefore in inflammatory processes. Extracellular proteases, many shed (or ripped, from a process called "ripping"[175]), which alter the chemokine gradients, participate in this crucial early step of the immune response. For CD26, the modulation of SDF-1 and the CXCR4 axis of cell homing has been particularly well studied[214, 215]; (2) Circulating sCD26 may also participate in the clipping or inactivation of the biologically still active blood substrates such as vascular regulatory peptides (substance P or bradykinin)[216-224], growth factors or hormones (e.g. only 20% of incretins GLP-1 and GIP, which originated in the gastrointestinal duct, are still active in the blood pool)[139,140] and (3) In the case of oncogenic processes, in addition to possible involvement in both immunosuppressor[122,136] and angiogenic mechanisms[122,136], the process of shedding may initiate or dampen CD26 involvement in cell-adhesion processes through fibronectin, ADA or collagen binding[121,123-126,144,154,225-227].

sCD26 PATHOPHYSIOLOGY AND CRC DIAGNOSIS

Many studies have demonstrated altered serum levels of enzymatic DPP-IV activity (see review[142]) and soluble CD26 protein in several diseases. Some studies show contradictory results, probably related to the stage of the disease considered (or in which a particular patient has been recruited)[103,110,111,158, 228-230]. However, other discrepancies between enzymatic activity and protein concentration measurements can be explained by putative changes in the glycosylation pattern (leading to a lack of immunorecognition of sCD26), the putative presence of the DPP-IV activator attractin, inhibitor glypican-3 or the secretion of other dipeptidyl peptidases such as DPP-II or soluble FAPα (DASH). For example, in myocardial infarction patients treated with streptokinase, the enzyme concentration is reduced to more than 50% after 90 d of therapy, while measurements of DPP-IV enzymatic activity did not change during that period[211]. On the contrary, the same authors found that there was no change in sCD26 concentrations between healthy donors and patients with rheumatoid arthritis and lupus erythematosus, although a lower enzymatic activity was detected[158].

Reference values of DPP-IV specific activity show no differences in serum and plasma[118,142,187], but most reports do not use the same assay conditions or the same definition of specific activity -the same applies to the units of catalytic activity-, making it difficult to compare results even from the same authors. However, the amount of sCD26 antigen found in normal serum with the most commonly used commercial ELISA kit (Bender MedSystems), corresponds well with the expected values based on the specific activity of purified serum DPP-IV[118,142]. Together, these findings support the use of immunodetection techniques for the quantification of these molecules because they are more specific.

DPP-IV enzymatic activity is high in patients with hepatic cancer, hepatitis, osteoporosis, cholestasis and other liver diseases. On the other hand, the mean DPP-IV activity remains unchanged in metastatic bone disease, esophagus, gall bladder, chronic myelocytic leukemia or leiomyosarcoma cancers, in allergic asthma, celiac disease,and adult T-cell leukemia, although serum DPP-IV in the latter is strongly correlated with the percentage of CD26+ T cells. However, decreased levels of DPP-IV were observed in patients with acute lymphocytic leukemia, thyroid and oral cancer, advanced gastric carcinoma, HCV infections, inflammatory bowel diseases, type II diabetes, in healthy smokers, in pregnancy, and in alcoholics and patients suffering from major depression.

Table 1 Performance characteristics of the sCD26 test

	Cohort *n*	CRC risk	sCD26 cut-off (ng/mL)	CRC in sCD26+ patients	Advanced adenomas in sCD26+ patients	Polyps in sCD26+ patients	Other findings in sCD26+ patients	Sensitivity (%)	Specificity (%)
1st Case-control study[89]	175	Diagnosed with CRC	410	99/110	-	-	6/110 Crohn´s 1/110 GC	90% (CRC)	90% (CRC)
1st Case-finding study[97]	170	Average-risk	410	-	-[a]	8/21	2/21 diverticula	-	-
2nd Case-finding study[95]	2673	Average- and increased-risk	410	2/140	4/140[2]	46/140[2]	12/140 diverticula	100% (CRC)[1]	89.9% (CRC)[1]
2nd Case-control study [94]	299	Increased-risk	460	27/110	20/110	13/110	18/110 IBD; 18/110 non-IBD; 14/110 anemia, diarrhea, rectal bleeding	81.8% (CRC) 58% (CN)	72.3% (CRC) 75.5 (CN)

n: Number of individuals; CR: Colorectal; CRC: Colorectal cancer; CN: Colorectal neoplasms including CRC and advanced adenomas; GC: Gastric cancer; IBD (inflammatory bowel diseases, includes colitis and Crohn's); non-IBD (includes hemorrhoids and diverticula). [a]No data on polyps´ pathology could be obtained; [1]: Data obtained after one-year follow-up for the detection of interval cancers. [2]: Pathological anatomy information obtained only for 16 of 46 polyps. The four advanced adenomas are also included in the polyps' column.

A reduction in DPP-IV activity has been related to symptoms of depression and anxiety under certain circumstances. Contradictory results were reported for psychologically-related eating disorders such as anorexia or bulimia, CRC, rheumatoid arthritis, lupus erythematosus and Sjögren syndrome.

Many studies have used sCD26 as a soluble marker of Th1 cellular immune activation, together with sCD30 and sometimes sCD23 as markers of Th2 (humoral response)[231-234]. The concentration of sCD26 increases in HIV-1 patients, leishmaniasis, myocardial infarction and atopic dermatitis. It does not change in asthmatics, osteoarthritis and gastric cancers. In many, but not all studies, it decreases in rheumatoid arthritis and particularly in lupus erythematosus and Sjögren syndrome, while results from hepatitis C virus (HCV) are not consistent. In summary, low levels of DPP-IV/sCD26 occur concurrently with impaired immune status -some hematological and solid malignancies can be included-, whereas increased levels occur in inflammatory and infectious diseases (enhanced immune status), other hematological tumors, and liver diseases[142].

We were the first to report reduced levels of sCD26, using immunodetection, in the serum of CRC patients, compared with healthy donors[103]. Reduced levels of enzymatic activity were reported for a small group of patients in 1987[235], although other authors found increased DPP-IV activity in a cohort of CRC patients comparable to ours[229]. We have already made some putative explanations to clarify this lack of correlation. From a biological point of view, further research studies are needed, but this issue does not affect the focus of this article. Our most important finding was that lower concentrations were found particularly in the early stages of the disease. Sensitivities higher than 80% (Table 1) were found for Dukes' stages A, B and C, whereas it was impaired in Dukes' stage D, in which CEA levels diagnosed better. Interestingly, it was in stage D where the DPP-IV activity actually increased in the study mentioned[229]. In this first study, we also found that sCD26 as a variable is not related with Dukes' stage classification, age, gender, tumor location or degree of differentiation, which also suggested the potential usefulness of this molecule for early diagnosis of CRC. We also showed preliminary data on the potential prognostic value with a follow-up of 2 years until recurrence; additional data has not yet been published. Moreover, we did not find changes in related diseases such as gastric-tract carcinomas, and in two of four blood cell cancers the concentration was raised (n = 4); impaired levels of sCD26 were observed only in some cases with gastric tract benign pathology and with Crohn's disease[103, 228].

These last results on specificity agreed with published works, and Crohn's disease data may be irrelevant for screening since these patients should have been detected years before the CRC screening procedure. To establish the feasibility of the sCD26 test for the diagnosis of CRC, we decided to perform a first pilot case-finding study that tested 170 persons of both genders at average risk for CRC (older than 50 years and asymptomatic for bowel disease), excluding individuals with a family history of CRC, or colorectal polyps, or personal history of CRC. From 29 individuals positive for the marker (with serum levels below or at the cut-off of 410 ng/mL), as previously studied[113], 21 underwent the colonoscopic procedure with colorectal findings in ten individuals (47.6%) against 3 out of ten individuals negative for the marker (30%)[113], showing an additional value of sCD26 for the detection of premalignant lesions (Table 1).

The aim of a later case-finding study in a large cohort (2754 presumably healthy individuals) was to evaluate its association with epidemiologic parameters as well as certain common digestive-related symptoms or pathologies[109]. Personal questionnaires were completed for data such as personal and familial history of colorectal polyps or cancer, bowel diseases (non-inflammatory benign pathologies: anal fissure, hemorrhoids, diverticula, irritable bowel syndrome and spastic colon; and inflammatory bowel diseases: colitis

or Crohn's disease), symptoms (rectal bleeding or fecal blood and changes in bowel habits), and smoking status. Individuals with a personal history of CRC, personal history of a cancer other than CRC, personal history of colorectal polyps, and familial history of cancer and/or colorectal polyps were excluded.

The mean sCD26 concentration in this cohort corresponded to 555.9 ± 181.7 ng/mL, similar to that previously reported for 52 healthy donors (559.7 ± 125.5 ng/mL). However, the range in this large cohort was considerably broad compared to that of the healthy donors (118–3062 ng/mL and 273–863 ng/mL, respectively)[109]. Information concerning the smoking status was also obtained. 63.8% of the individuals were non-smokers, 27.8% were current smokers, while 8.4% were former smokers. Former smokers showed statistically significant higher values of sCD26, and current smokers lower than non-smokers, the latter fact correlating with data on enzymatic activity[236-240], however, this small difference (20 and 10 ng/mL, respectively) was not statistically significant when grouped by the number of cigarettes per day.

According to the cut-off point 410 ng/mL, 273 individuals (10.2%) were sCD26+[109]. To extend the validation of sCD26 as an early biomarker for CRC, a colonoscopic procedure was recommended to these individuals. Among the 140 individuals that underwent colonoscopy, one case of CRC was diagnosed, resulting in a very high prevalence (0.7%) for this cohort. In addition, there were 46 cases of colorectal polyps (32.9%), 12 cases of colorectal diverticula (8.6%) and 81 individuals without apparent colorectal pathology (Table 1). The PPV for the sCD26 test considering all the findings was 42.1%[111]. The sCD26+ individual diagnosed with CRC after colonoscopy received surgery after three months, finding a tumor in Dukes' stage B. Interestingly this patient had a negative FOBT two weeks before the measurement of sCD26. Another case was a sCD26+ individual who had a second positive test three months afterwards, and later was diagnosed by colonoscopy with a 1-cm villous polyp in the transverse colon, which was not extirpated. After seven months, the patient was diagnosed and operated on for a moderately differentiated adenocarcinoma at Dukes' stage A (data not published). The 46 individuals diagnosed with colorectal polyps represent a percentage similar to those diagnosed in the first case-finding study, also elevated considering the average risk[30]. Information regarding the pathological anatomy of polyps was obtained only for 16 cases. Of these, 75% presented neoplasic histology (adenomas). Trying to explain the high number of colorectal polyps diagnosed, no differences were found between the mean age of the individuals with and without polyps (data not published).

Although the most accurate means of measuring sensitivity and specificity is to perform colonoscopies in all the screened patients regardless of the test result, when this is not possible, several authors use a follow-up period to detect interval cancers[24,214,241]. Therefore, it is assumed that a false negative becomes clinically apparent through subsequent screening or the appearance of symptoms. According to this approach, with a one year follow-up of our individuals, a sensitivity of 100% and a specificity of 89.9% for CRC were obtained (Table 1)[111].

These results, with special interest on the absence of correlation among all the parameters analyzed, particularly the personal and familial history of CRC and polyps together with rectal bleeding and changes in bowel habits, proved that the sCD26 test can be easily offered and evaluated in a large population cohort. Additional data also support the usefulness of serum sCD26 levels for patient monitoring because four of the patients diagnosed with polyps requested a second sCD26 test after polypectomy, which showed normalized values (> 410 ng/mL) in the new measurement in all patients[111].

However, accurate clinical values suggesting that a serum CD26 test is an improvement on the current non-invasive screening tests recommended was lacking. Therefore a case-control study with 299 symptomatic and asymptomatic patients, who were to undergo colonoscopy, was performed[108]. Colonoscopy indication was mostly due to symptoms such as rectal bleeding, abdominal pain, diarrhea, constipation, anemia, colorectal polyp or cancer surveillance, and CRC screening. Patients were classified into groups as follows: no colorectal pathology (symptomatic with rectal bleeding, abdominal pain, diarrhea, anemia, constipation, or asymptomatic with personal history of polyps or CRC, and family history of polyps); non-IBD (hemorrhoids and diverticula); IBD (colitis or Crohn's disease); colorectal polyps (hyperplastic polyps, non-advanced adenomas and advanced adenomas); and CRC.

The average sCD26 level for the group of patients with no colorectal pathology or benign colorectal pathology was 641.2 ± 241.2 ng/mL, higher than the cut-off point obtained with healthy donors as the control cohort. Therefore, we chose to calculate a new cut-off, 460 ng/mL. According to this, the sCD26 test has a sensitivity and specificity of 81.8% and 72.3%, respectively, for CRC (Table 1), (a specificity of 79.3% when the group of symptomatic patients with no colorectal pathology was considered). The mean sCD26 concentration decreased, although non-significantly, as the pathology diagnosed was more severe, that is, from no colorectal pathology to CRC, with a noticeable decrease in the group with IBD and anemia. Interestingly, individuals with anemia showed a substantially elevated sCD26 positivity rate (71.4%), as well as the IBD group (69.2%), both responsible for the specificity value. IBD is associated with at least a 5-fold increased risk for CRC, representing one of the highest risk groups based on the inflammation-dysplasia-carcinoma sequence[242]. However, these individuals are usually diagnosed at the age of the potential CRC screening procedure, as commented, and its impact on the specificity data can be avoided. When considering only asymptomatic individuals, specificity increases to 90%, which agrees with our previously published results[103,104,113].

On the other hand, as no carcinomas *in situ* were detected in the patients included in the study, the decrease in sensitivity in this context is probably related to altered frequencies in CRC stages (sCD26 is a poorer marker in Duke's A than in B or C stages)[103].

In this study, we also analyzed the relationship of this biomarker with advanced adenomas. Defining advanced adenomas as those larger than 10 mm, with tubulovillous or villous histology, or with high-grade dysplasia, and classifying patients with more than one polyp according to the most advanced lesion, sensitivity for the detection of CRC and advanced adenomas was 58.0%, with a specificity of 75.5% (Table 1). We found no statistical differences, according to the sCD26 positivity rate, with regard to the number of polyps, their size, location, morphology or histology, but differences closely significant were observed with the grade of dysplasia, a morphological marker of neoplastic lesions. The positivity rate increased gradually with the degree of dysplasia: 22.2% for non-dysplastic polyps, 32.5% for low-grade dysplastic adenomas and almost double (60.0%) for high-grade dysplastic adenomas. Concerning advanced adenomas, a term commonly used to group adenomas that have an increased likelihood of malignant transformation, the sCD26 positivity rate was statistically significant.

As commented, iFOBT is now preferentially offered for average-risk screening. A highly sensitive FOBT (guaiac-based) test (Hemoccult SENSA®) reached 71-79% sensitivity with single testing, and 85% with multiple testing, with corresponding specificities of 86% and 95%[241-243], although these parameters are probably overestimated as these studies lacked colonoscopic examination of the negative cases. For both pathologies together (CRC and advanced adenomas) in an asymptomatic high-risk cohort, however, higher sensitivity, 65.3% (and 87.5% for specificity), resulted with Hemoccult SENSA®[244] compared to iFOGT (33.1% sensitivity and specificity of 97.5%, parameters obtained with flexible sigmoidoscopy). For other experimental serum biomarkers, the CCSA-2 has shown 97.3% sensitivity and 78.4% specificity, although hyperplastic polyps and non-advanced adenomas were considered as findings, while IBD patients were absent in their cohort[100]. Therefore, sCD26 seems to perform adequately as a blood biomarker for CRC and advanced adenomas, and is independent of the frequent but intermittent bleeding, unlike guaiac FOBT or iFOBT.

In hepatocarcinoma, a loss of membrane CD26 is correlated with higher DPP-IV levels. This fact is not seen in CRC, as almost all CRC patients show reduced serum levels of sCD26[103,111], but loss of membrane CD26 expression only occurs in 11% of colorectal tumor[181]. In conclusion, for CRC, sCD26 is not correlated with cell proliferation, or with the alteration of CD26 expression in CRC tumor cells. Nor is there any direct correlation between sCD26 levels and tumor location, degree of histological differentiation, type of metastasis or Dukes' stages of CRC[245], which may affect the hepatic production of sCD26. Therefore, sCD26 is also independent, if not of the tumorigenic locus, at least of the tumorigenic tissue. In addition, as it seems immune-related[142], the sCD26 decrease in the plasma of patients should appear sooner in the adenoma-carcinoma development compared to the presence of fecal blood.

CONCLUSION

As commented previously, it has recently been reported that screening accounted for 53% of the decline in CRC mortality observed during 1975-2000 in the USA (26% less mortality). Moreover, decline in CRC mortality in the USA could be enhanced if current trends against cancer were accelerated[2]. Therefore, any kind of screening strategy should be proposed in advanced and developing countries. For FOBT Hemoccult, which is a non-invasive and relatively cheap test, there are considerable data from many prospective studies in different countries of the world; however, not many countries include this screening method in their public health systems.

In the context of this review, dealing with an experimental CRC screening test that is easier to monitor in the health system, or with a better clinical value, the idea of comparing different tests rather than conducting long-term and expensive randomized controlled trials to evaluate each new test is very important, as suggested by Mandel[37] in the commentary on the Allison study that compared performance, outcome, compliance, and cost of guaiac FOBT and iFOBT[42] fecal occult blood tests. In this way, it will be much easier to study and promote new fecal or blood tests. With this aim, we are currently initiating a multicentric, prospective, double-blinded study in an average-risk population, where the performance of the quantitive iFOBT and the sCD26 assay will be assessed and compared with the gold standard colonoscopy.

Another important idea, as we have previously proposed[110], is the combination of biomarkers for the management of cancer, since it is difficult to achieve a simple test to detect early-stage tumors that is useful for screening purposes. For example, we have tested sCD26 levels, α-L-fucosidase activity and CEA in the same patients[109], and while, at a specificity of 100%, α-L-fucosidase activity did not enhance the sensitivity value obtained with sCD26 alone in TNM stage II patients, the sensitivity obtained from the combination of both markers was 65% *versus* 33% for sCD26 alone in TNM stage I patients. In the same way, a very recent work assessed the combination of CEA with three other biomarkers, sCD26, DR-70 and MMP-9, previously selected from 26 candidates, for the detection of CRC[246]. This study confirmed that sCD26 and DR-70 (fibrin and fibrinogen degradation products[104]) are the more promising of the available serum markers, although DR-70 showed a significant correlation with age. Values of the area under the ROC curve, and sensitivity and specificity for sCD26 were similar to our latest study mentioned above[108] using a similar cohort of case-control patients who attended

colonoscopy. The same study[246] also showed that a combination of sCD26, DR-70 and CEA detected CRC, particularly at the early stage of disease, significantly better than CEA alone or other biomarker combinations at certain specificities[246]. Nevertheless, our data on sCD26 for the detection of the earliest stages were much better (and data of CEA worst)[103,109]. This discrepancy perhaps may be due to the differences in the cohort composition in each study, to the statistical method employed for the combination of biomarkers, or to a development of the ELISA for the measurement of CEA levels (the kits used for CEA, but not for sCD26, were different in each study).

Therefore, the approach tries to increase the clinical value of each biomarker or yield a test more able to distinguish between patients and healthy individuals, and ideally also among different kinds of tumors, in the way we have tested for head and neck cancer *versus* non-small cell lung cancer[247] at a low scale, and others for the screening of Alzheimer disease[248], or to identify lymph node metastases in non-small cell lung cancer patients[249]. In this case, a panel of six serum biomarkers classified the patients better than conventional clinical methods.

To measure several biomarkers at a time, ELISAs for key serum markers are being arrayed or multiplexed based on immunoblot technology or flow cytometric beads[110,247,249].

These techniques, in relation with other genomic or proteomic techniques, are more transferable to practical application in clinical decision-making. In this sense, it is interesting to note that the multiplexed diagnostics market has grown rapidly and generated sales of approximately $2.4 billon in 2009, and is expected to tip in favor of continued rapid growth, reaching almost $5.8 billion in 2015. Moreover, the multivariate data obtained from such a test can easily be managed with new statistical methods already developed for the fields of genomics and proteomics in general.

However, as multiple cancer screening tests are been advocated for the general population, clinicians and patients are not always well-informed of screening burdens. For example, in the ongoing Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, a randomized controlled trial to determine the effects of prostate, lung, colorectal, and ovarian cancer screening on disease-specific mortality, an individual has an approximately 50% or greater risk of a false-positive finding by the 14th test[250]. Physicians should educate patients about the likelihood of false positives and resulting diagnostic interventions when counseling on cancer screening.

REFERENCES

1 **Jemal A**, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin* 2007 ; **57**: 43-66

2 **Center MM**, Jemal A, Ward E. International trends in colorectal cancer incidence rates. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1688-1694

3 **Umar A**, Greenwald P. Alarming colorectal cancer incidence trends: a case for early detection and prevention. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1672-1673

4 **Bond JH**. Clinical evidence for the adenoma-carcinoma sequence, and the management of patients with colorectal adenomas. *Semin Gastrointest Dis* 2000; **11**: 176-184

5 **Leslie A**, Carey FA, Pratt NR, Steele RJ. The colorectal adenoma-carcinoma sequence. *Br J Surg* 2002; **89**: 845-860

6 **Atkin WS**, Morson BC, Cuzick J. Long-term risk of colorectal cancer after excision of rectosigmoid adenomas. *N Engl J Med* 1992; **326**: 658-662

7 **Mandel JS**, Church TR, Ederer F, Bond JH. Colorectal cancer mortality: effectiveness of biennial screening for fecal occult blood. *J Natl Cancer Inst* 1999; **91**: 434-437

8 **Edwards BK**, Ward E, Kohler BA, Eheman C, Zauber AG, Anderson RN, Jemal A, Schymura MJ, Lansdorp-Vogelaar I, Seeff LC, van Ballegooijen M, Goede SL, Ries LA. Annual report to the nation on the status of cancer, 1975-2006, featuring colorectal cancer trends and impact of interventions (risk factors, screening, and treatment) to reduce future rates. *Cancer* 2010; **116**: 544-573

9 **Smith RA**, Cokkinides V, Eyre HJ. American Cancer Society guidelines for the early detection of cancer, 2006. *CA Cancer J Clin* 2006; **56**: 11-25; quiz 49-50

10 **U.S. Preventive Services Task Force.** Guide to Clinical Preventive Services 2009, Recommendations of the U.S. Preventive Services Task Force 2009. http: //www.ahrq.gov/clinic/pocketgd.htm

11 Council of the European Union Recommendation of 2 December 2003 on cancer screening. *OJEU* 2003; **L327**: 34-38

12 **Winawer SJ**. Screening of colorectal cancer. *Surg Oncol Clin N Am* 2005; **14**: 699-722

13 **Mandel JS**, Bond JH, Church TR, Snover DC, Bradley GM, Schuman LM, Ederer F. Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. *N Engl J Med* 1993; **328**: 1365-1371

14 **Hawk** ET, Levin B. Colorectal cancer prevention. *J Clin Oncol* 2005; **23**: 378-391

15 **Smith RA**, Cokkinides V, von Eschenbach AC, Levin B, Cohen C, Runowicz CD, Sener S, Saslow D, Eyre HJ. American Cancer Society guidelines for the early detection of cancer. *CA Cancer J Clin* 2002; **52**: 8-22

16 **Vijan S**, Hwang EW, Hofer TP, Hayward RA. Which colon cancer screening test? A comparison of costs, effectiveness, and compliance. *Am J Med* 2001; **111**: 593-601

17 **Weissfeld JL**, Schoen RE, Pinsky PF, Bresalier RS, Church T, Yurgalevitch S, Austin JH, Prorok PC, Gohagan JK. Flexible Sigmoidoscopy in the PLCO Cancer Screening Trial: Results From the Baseline Screening Examination of a Randomized Trial. *J Natl Cancer Inst* 2005; **97**: 989-997

18 **Pignone M**. Is population screening for colorectal cancer cost-effective?. *Nat Clin Pract Gastroenterol Hepatol* 2005; **2**: 288-289

19 U.S. Preventive Services Task Force. Screening for colorectal cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2008; **149**: 627-637

20 **Seeff LC**, Richards TB, Shapiro JA, Nadel MR, Manninen DL, Given LS, Dong FB, Winges LD, McKenna MT. How many endoscopies are performed for colorectal cancer screening? Results from CDC's survey of endoscopic capacity. *Gastroenterology* 2004; **127**: 1670-1677

21 **Seeff LC**, Nadel MR, Klabunde CN, Thompson T, Shapiro JA, Vernon SW, Coates RJ. Patterns and predictors of colorectal cancer test use in the adult U.S. population. *Cancer* 2004; **100**: 2093-2103

22 **Klabunde CN**, Lanier D, Nadel MR, McLeod C, Yuan G, Vernon SW. Colorectal cancer screening by primary care physicians: recommendations and practices, 2006-2007. *Am J Prev Med* 2009; **37**: 8-16

23 **Warren JL**, Klabunde CN, Mariotto AB, Meekins A, Topor M, Brown ML, Ransohoff DF. Adverse events after outpatient colonoscopy in the Medicare population. *Ann Intern Med* 2009; **150**: 849-57, W152

24 **Kronborg O**, Fenger C, Olsen J, Jørgensen OD, Søndergaard O. Randomised study of screening for colorectal cancer with faecal-occult-blood test. *Lancet* 1996; **348**: 1467-1471

25 **Hardcastle JD**, Chamberlain JO, Robinson MH, Moss SM, Amar SS, Balfour TW, James PD, Mangham CM. Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. *Lancet* 1996; **348**: 1472-1477

26 **Winawer S**, Faivre J, Selby J, Bertaro L, Chen TH, Kroborg O, Levin B, Mandel J, O'Morain C, Richards M, Rennert G, Russo A, Saito H, Semigfnovsky B, Wong B, Smith R. Workgroup II: the screening process. UICC International Workshop on Facilitating Screening for Colorectal Cancer, Oslo, Norway (29 and 30 June 2002). *Ann Oncol* 2005; **16**: 31-33

27 **Cole SR**, Young GP, Esterman A, Cadd B, Morcom J. A randomised trial of the impact of new faecal haemoglobin test technologies on population participation in screening for colorectal cancer. *J Med Screen* 2003; 10: 117-122

28 **Trinite T**, Loveland-Cherry C, Marion L. The U.S. Preventive Services Task Force: an evidence-based prevention resource for nurse practitioners. *J Am Acad Nurse Pract* 2009; **21**: 301-306

29 **Huang CS**, Lal SK, Farraye FA. Colorectal cancer screening in average risk individuals. *Cancer Causes Control* 2005; **16**: 171-188

30 **Imperiale TF**, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med* 2004; **351**: 2704-2714

31 **Fraser CG**, Matthew CM, Mowat NA, Wilson JA, Carey FA, Steele RJ. Immunochemical testing of individuals positive for guaiac faecal occult blood test in a screening programme for colorectal cancer: an observational study. *Lancet Oncol* 2006; **7**: 127-131

32 **Smith A**, Young GP, Cole SR, Bampton P. Comparison of a brush-sampling fecal immunochemical test for hemoglobin with a sensitive guaiac-based fecal occult blood test in detection of colorectal neoplasia. *Cancer* 2006; **107**: 2152-2159

33 **Young GP**, St John DJ, Winawer SJ, Rozen P. Choice of fecal occult blood tests for colorectal cancer screening: recommendations based on performance characteristics in population studies: a WHO (World Health Organization) and OMED (World Organization for Digestive Endoscopy) report. *Am J Gastroenterol* 2002; **97**: 2499-2507

34 **Federici A**, Giorgi Rossi P, Borgia P, Bartolozzi F, Farchi S, Gausticchi G. The immunochemical faecal occult blood test leads to higher compliance than the guaiac for colorectal cancer screening programmes: a cluster randomized controlled trial. *J Med Screen* 2005; **12**: 83-88

35 **Saito H**, Soma Y, Nakajima M, Koeda J, Kawaguchi H, Kakizaki R, Chiba R, Aisawa T, Munakata A. A case-control study evaluating occult blood screening for colorectal cancer with hemoccult test and an immunochemical hemagglutination test. *Oncol Rep* 2000; **7**: 815-819

36 **Levi Z**, Rozen P, Hazazi R, Vilkin A, Waked A, Maoz E, Birkenfeld S, Leshno M, Niv Y. A quantitative immunochemical fecal occult blood test for colorectal neoplasia. *Ann Intern Med* 2007; 146: 244-255

37 **Mandel JS**. Which colorectal cancer screening test is best? *J Natl Cancer Inst* 2007; **99**: 1424-1425

38 **Saito H**, Soma Y, Koeda J, Wada T, Kawaguchi H, Sobue T, Aisawa T, Yoshida Y. Reduction in risk of mortality from colorectal cancer by fecal occult blood screening with immunochemical hemagglutination test. A case-control study. *Int J Cancer* 1995; **61**: 465-469

39 **Nakajima M**, Saito H, Soma Y, Sobue T, Tanaka M, Munakata A. Prevention of advanced colorectal cancer by screening using the immunochemical faecal occult blood test: a case-control study. *Br J Cancer* 2003; **89**: 23-28

40 **Saito H**. Colorectal cancer screening using immunochemical faecal occult blood testing in Japan. *J Med Screen* 2006; **13** Suppl 1: S6-S7

41 **Lee KJ**, Inoue M, Otani T, Iwasaki M, Sasazuki S, Tsugane S. Colorectal cancer screening using fecal occult blood test and subsequent risk of colorectal cancer: a prospective cohort study in Japan. *Cancer Detect Prev* 2007; **31**: 3-11

42 **Allison JE**, Sakoda LC, Levin TR, Tucker JP, Tekawa IS, Cuff T, Pauly MP, Shlager L, Palitz AM, Zhao WK, Schwartz JS, Ransohoff DF, Selby JV. Screening for colorectal neoplasms with new fecal occult blood tests: update on performance characteristics. *J Natl Cancer Inst* 2007; **99**: 1462-1470

43 **Karl J**, Wild N, Tacke M, Andres H, Garczarek U, Rollinger W, Zolg W. Improved diagnosis of colorectal cancer using a combination of fecal occult blood and novel fecal protein markers. *Clin Gastroenterol Hepatol* 2008; **6**: 1122-1128

44 **Ahlquist DA**, Skoletsky JE, Boynton KA, Harrington JJ, Mahoney DW, Pierceall WE, Thibodeau SN, Shuber AP. Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. *Gastroenterology* 2000; **119**: 1219-1227

45 **Hanash SM**, Baik CS, Kallioniemi O. Emerging molecular biomarkers--blood-based strategies to detect and monitor cancer. *Nat Rev Clin Oncol* 2011; 8: 142-150

46 **Tjalsma H**. Identification of biomarkers for colorectal cancer through proteomics-based approaches. *Expert Rev Proteomics* 2010; **7**: 879-895

47 **Hundt S**, Haug U, Brenner H. Blood markers for early detection of colorectal cancer: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 1935-1953

48 **Tataryn DN**, MacFarlane JK, Murray D, Thomson DM. Tube leukocyte adherence inhibition (LAI) assay in gastrointestinal (GIT) cancer. *Cancer* 1979; **43**: 898-912

49 **Liu HP**, Yan ZS, Zhang SS. The application of leukocyte adherence inhibition assay to patients with colorectal cancer. Comparison with serum level of carcinoembryonic antigen and sialic acid. *Dis Colon Rectum* 1989; **32**: 210-213

50 **Wang JY**, Yeh CS, Chen YF, Wu CH, Hsieh JS, Huang TJ, Huang SY, Lin SR. Development and evaluation of a colorimetric membrane-array method for the detection of circulating tumor cells in the peripheral blood of Taiwanese patients with colorectal cancer. *Int J Mol Med* 2006; **17**: 737-747

51 **Wilson L**, Cross S, Gimzewski J, Rao J. Nanocytology: a novel class of biomarkers for cancer management. *IDrugs* 2010; **13**: 847-851

52 **Kopreski MS**, Benko FA, Borys DJ, Khan A, McGarrity TJ, Gocke CD. Somatic mutation screening: identification of individuals harboring K-ras mutations with the use of plasma DNA. *J Natl Cancer Inst* 2000; **92**: 918-923

53 **Wang JY**, Hsieh JS, Chen CC, Tzou WS, Cheng TL, Chen FM, Huang TJ, Huang YS, Huang SY, Yang T, Lin SR. Alterations of APC, c-met, and p53 genes in tumor tissue and serum of patients with gastric cancers. *J Surg Res* 2004; **120**: 242-248

54 **Leung WK**, To KF, Man EP, Chan MW, Bai AH, Hui AJ, Chan FK, Sung JJ. Quantitative detection of promoter hypermethylation in multiple genes in the serum of patients with colorectal cancer. *Am J Gastroenterol* 2005; **100**: 2274-2279

55 **Umetani N**, Kim J, Hiramatsu S, Reber HA, Hines OJ, Bilchik AJ, Hoon DS. Increased integrity of free circulating DNA in sera of patients with colorectal or periampullary cancer: direct quantitative PCR for ALU repeats. *Clin Chem* 2006; **52**: 1062-1069

56 **Kelley RK**, Van Bebber SL, Phillips KA, Venook AP. Personalized medicine and oncology practice guidelines: a case study of contemporary biomarkers in colorectal cancer. *J Natl Compr Canc Netw* 2011; **9**: 13-25

57 **Asghar U**, Hawkes E, Cunningham D. Predictive and prognostic biomarkers for targeted therapy in metastatic colorectal cancer. *Clin Colorectal Cancer* 2010; **9**: 274-281

58 **Newton KF**, Newman W, Hill J. Review of Biomarkers in

Colorectal Cancer. *Colorectal Dis* 2010; [Epub ahead of print]

59 **Deng D**, Liu Z, Du Y. Epigenetic alterations as cancer diagnostic, prognostic, and predictive biomarkers. *Adv Genet* 2010; **71**: 125-176

60 **Pritchard CC**, Grady WM. Colorectal cancer molecular biology moves into clinical practice. *Gut* 2011; **60**: 116-129

61 **Bustin SA**, Gyselman VG, Williams NS, Dorudi S. Detection of cytokeratins 19/20 and guanylyl cyclase C in peripheral blood of colorectal cancer patients. *Br J Cancer* 1999; **79**: 1813-1820

62 **Liu M**, Chen H. The role of microRNAs in colorectal cancer. *J Genet Genomics* 2010; **37**: 347-358

63 **Heneghan HM**, Miller N, Kerin MJ. Systemic microRNAs: novel biomarkers for colorectal and other cancers? *Gut* 2010; **59**: 1002-104; author reply 1004

64 **Manne U**, Shanmugam C, Bovell L, Katkoori VR, Bumpers HL. miRNAs as biomarkers for management of patients with colorectal cancer. *Biomark Med* 2010; **4**: 761-770

65 **Mitchell PS**, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A* 2008; **105**: 10513-10518

66 **Dong Y**, Wu WK, Wu CW, Sung JJ, Yu J, Ng SS. MicroRNA dysregulation in colorectal cancer: a clinical perspective. *Br J Cancer* 2011; **104**: 893-898

67 **Huang Z**, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. *Int J Cancer* 2010; **127**: 118-126

68 **Thomson DM**, Krupey J, Freedman SO, Gold P. The radioimmunoassay of circulating carcinoembryonic antigen of the human digestive system. *Proc Natl Acad Sci U S A* 1969; **64**: 161-167

69 **Huber K**, Kirchheimer JC, Sedlmayer A, Bell C, Ermler D, Binder BR. Clinical value of determination of urokinase-type plasminogen activator antigen in plasma for detection of colorectal cancer: comparison with circulating tumor-associated antigens CA 19-9 and carcinoembryonic antigen. *Cancer Res* 1993; **53**: 1788-1793

70 **Fernandes LC**, Kim SB, Matos D. Cytokeratins and carcinoembryonic antigen in diagnosis, staging and prognosis of colorectal adenocarcinoma. *World J Gastroenterol* 2005; **11**: 645-648

71 **Herlyn M**, Blaszczyk M, Bennicelli J, Sears HF, Ernst C, Ross AH, Koprowski H. Selection of monoclonal antibodies detecting serodiagnostic human tumor markers. *J Immunol Methods* 1985; **80**: 107-116

72 **Kawahara M**, Chia D, Terasaki PI, Roumanas A, Sugich L, Hermes M, Iguro T. Detection of sialylated LewisX antigen in cancer sera using a sandwich radioimmunoassay. *Int J Cancer* 1985; 36: 421-425

73 **Arai M**, Sakamoto K, Otsuka H, Yokoyama Y, Akagi M. Detection of tumor associated antigen, PA8-15, in sera from pancreatic and gastrointestinal carcinoma patients. *Jpn J Clin Oncol* 1990; **20**: 145-153

74 **Pinczower GD**, Gianello RD, Williams RP, Preston BN, Preston H, Linnane AW. Monoclonal antibody 4D3 detects small intestinal mucin antigen (SIMA)--glycoprotein in the serum of patients with colorectal cancer. *Int J Cancer* 1993; **54**: 391-396

75 **Kuroki M**, Matsushita H, Matsumoto H, Hirose Y, Senba T, Yamamoto T. Nonspecific cross-reacting antigen-50/90 (NCA-50/90) as a new tumor marker. *Anticancer Res* 1999; **19**: 5599-5606

76 **Eskelinen M**, Pasanen P, Janatuinen E, Pettersson N, Linnane A, Alhava E. Small intestinal mucin antigen (SIMA); a novel tumour marker in colorectal cancer? *Anticancer Res* 1995 ; **15**: 2351-2356

77 **Duraker N**, Can D, Parilti M. Measurement of serum total and free prostate-specific antigen in women with colorectal carcinoma. *Br J Cancer* 2002; **86**: 203-206

78 **Yamaguchi A**, Kurosaka Y, Ishida T, Nishimura G, Kanno M, Kosaka T, Yonemura Y, Miyazaki I. Clinical significance of tumor marker NCC-ST 439 in large bowel cancers. *Dis Colon Rectum* 1991; **34**: 921-924

79 **Hammel P**, Boissier B, Chaumette MT, Piedbois P, Rotman N, Kouyoumdjian JC, Lubin R, Delchier JC, Soussi T. Detection and monitoring of serum p53 antibodies in patients with colorectal cancer. *Gut* 1997; **40**: 356-361

80 **Broll R**, Duchrow M, Oevermann E, Wellm C, Schwandner O, Schimmelpenning H, Roblick UJ, Bruch HP, Windhövel U. p53 autoantibodies in sera of patients with a colorectal cancer and their association to p53 protein concentration and p53 immunohistochemistry in tumor tissue. *Int J Colorectal Dis* 2001; **16**: 22-27

81 **Chang SC**, Lin JK, Lin TC, Liang WY. Genetic alteration of p53, but not overexpression of intratumoral p53 protein, or serum p53 antibody is a prognostic factor in sporadic colorectal adenocarcinoma. *Int J Oncol* 2005; **26**: 65-75

82 **Xia Q**, Kong XT, Zhang GA, Hou XJ, Qiang H, Zhong RQ. Proteomics-based identification of DEAD-box protein 48 as a novel autoantigen, a prospective serum marker for pancreatic cancer. *Biochem Biophys Res Commun* 2005; **330**: 526-532

83 **Reipert BM**, Tanneberger S, Pannetta A, Bedosti M, Poell M, Zimmermann K, Stellamor MT. Increase in autoantibodies against Fas (CD95) during carcinogenesis in the human colon: a hope for the immunoprevention of cancer? *Cancer Immunol Immunother* 2005; **54**: 1038-1042

84 **Hyodo I**, Doi T, Endo H, Hosokawa Y, Nishikawa Y, Tanimizu M, Jinno K, Kotani Y. Clinical significance of plasma vascular endothelial growth factor in gastrointestinal cancer. *Eur J Cancer* 1998; **34**: 2041-2045

85 **Kumar H**, Heer K, Lee PW, Duthie GS, MacDonald AW, Greenman J, Kerin MJ, Monson JR. Preoperative serum vascular endothelial growth factor can predict stage in colorectal cancer. *Clin Cancer Res* 1998; **4**: 1279-1285

86 **Broll R**, Erdmann H, Duchrow M, Oevermann E, Schwandner O, Markert U, Bruch HP, Windhövel U. Vascular endothelial growth factor (VEGF)--a valuable serum tumour marker in patients with colorectal cancer? *Eur J Surg Oncol* 2001; **27**: 37-42

87 **Tsai WS**, Changchien CR, Yeh CY, Chen JS, Tang R, Chiang JM, Hsieh PS, Fan CW, Wang JY. Preoperative plasma vascular endothelial growth factor but not nitrite is a useful complementary tumor marker in patients with colorectal cancer. *Dis Colon Rectum* 2006; **49**: 883-894

88 **Renehan AG**, Painter JE, O'Halloran D, Atkin WS, Potten CS, O'Dwyer ST, Shalet SM. Circulating insulin-like growth factor II and colorectal adenomas. *J Clin Endocrinol Metab* 2000; **85**: 3402-3408

89 **Mroczko B**, Szmitkowski M, Wereszczyńska-Siemiatkowska U, Okulczyk B. Stem cell factor (SCF) and interleukin 3 (IL-3) in the sera of patients with colorectal cancer. *Dig Dis Sci* 2005; **50**: 1019-1024

90 **Rabinovich GA**, Gabrilovich D, Sotomayor EM. Immunosuppressive strategies that are mediated by tumor cells. *Annu Rev Immunol* 2007; **25**: 267-296

91 **Melle C**, Ernst G, Schimmel B, Bleul A, Thieme H, Kaufmann R, Mothes H, Settmacher U, Claussen U, Halbhuber KJ, Von Eggeling F. Discovery and identification of alpha-defensins as low abundant, tumor-derived serum markers in colorectal cancer. *Gastroenterology* 2005; **129**: 66-73

92 **Roessler M**, Rollinger W, Palme S, Hagmann ML, Berndt P, Engel AM, Schneidinger B, Pfeffer M, Andres H, Karl J, Bodenmüller H, Rüschoff J, Henkel T, Rohr G, Rossol S, Rösch W, Langen H, Zolg W, Tacke M. Identification of

nicotinamide N-methyltransferase as a novel serum tumor marker for colorectal cancer. *Clin Cancer Res* 2005; **11**: 6550-6557
93 **Ayude D**, Fernández-Rodríguez J, Rodríguez-Berrocal FJ, Martínez-Zorzano VS, de Carlos A, Gil E, Páez de La Cadena M. Value of the serum alpha-L-fucosidase activity in the diagnosis of colorectal cancer. *Oncology* 2000; **59**: 310-316
94 **Zhang B**, Chen JY, Chen DD, Wang GB, Shen P. Tumor type M2 pyruvate kinase expression in gastric cancer, colorectal cancer and controls. *World J Gastroenterol* 2004; **10**: 1643-1646
95 **Matusiewicz M**, Krzystek-Korpacka M, Diakowska D, Grabowski K, Augoff K, Blachut K, Paradowski L, Kustrzeba-Wojcicka I, Piast M, Banas T. Serum sulfatase activity is more elevated in colonic adenomas than cancers. *Int J Colorectal Dis* 2008; **23**: 383-387
96 **Kumor A**, Daniel P, Pietruczuk M, Małecka-Panas E. Serum leptin, adiponectin, and resistin concentration in colorectal adenoma and carcinoma (CC) patients. *Int J Colorectal Dis* 2009; **24**: 275-281
97 **Konety BR**, Getzenberg RH. Nuclear structural proteins as biomarkers of cancer. *J Cell Biochem* 1999; Suppl 32-33: 183-191
98 **Leman ES**, Getzenberg RH. Nuclear structure as a source of cancer specific biomarkers. *J Cell Biochem* 2008; **104**: 1988-1993
99 **Leman ES**, Schoen RE, Weissfeld JL, Cannon GW, Sokoll LJ, Chan DW, Getzenberg RH. Initial analyses of colon cancer-specific antigen (CCSA)-3 and CCSA-4 as colorectal cancer-associated serum markers. *Cancer Res* 2007; **67**: 5600-5605
100 **Leman ES**, Schoen RE, Magheli A, Sokoll LJ, Chan DW, Getzenberg RH. Evaluation of colon cancer-specific antigen 2 as a potential serum marker for colorectal cancer. *Clin Cancer Res* 2008; **14**: 1349-1354
101 **Schneider J**, Bitterlich N, Schulze G. Improved sensitivity in the diagnosis of gastro-intestinal tumors by fuzzy logic-based tumor marker profiles including the tumor M2-PK. *Anticancer Res* 2005; **25**: 1507-1515
102 **Kozwich DL**, Kramer LC, Mielicki WP, Fotopoulos SS, Gordon SG. Application of cancer procoagulant as an early detection tumor marker. *Cancer* 1994; **74**: 1367-1376
103 **Cordero OJ**, Ayude D, Nogueira M, Rodriguez-Berrocal FJ, de la Cadena MP. Preoperative serum CD26 levels: diagnostic efficiency and predictive value for colorectal cancer. *Br J Cancer* 2000; **83**: 1139-1146
104 **Kerber A**, Trojan J, Herrlinger K, Zgouras D, Caspary WF, Braden B. The new DR-70 immunoassay detects cancer of the gastrointestinal tract: a validation study. *Aliment Pharmacol Ther* 2004; **20**: 983-987
105 **Soroush AR**, Zadeh HM, Moemeni M, Shakiba B, Elmi S. Plasma prolactin in patients with colorectal cancer. *BMC Cancer* 2004; **4**: 97
106 **Saito N**, Kameoka S. Serum laminin is an independent prognostic factor in colorectal cancer. *Int J Colorectal Dis* 2005; **20**: 238-244
107 **Fedarko NS**, Jain A, Karadag A, Van Eman MR, Fisher LW. Elevated serum bone sialoprotein and osteopontin in colon, breast, prostate, and lung cancer. *Clin Cancer Res* 2001; **7**: 4060-4066
108 **De Chiara L**, Rodríguez-Piñeiro AM, Rodríguez-Berrocal FJ, Cordero OJ, Martínez-Ares D, Páez de la Cadena M. Serum CD26 is related to histopathological polyp traits and behaves as a marker for colorectal cancer and advanced adenomas. *BMC Cancer* 2010; **10**: 333
109 **De Chiara L**, Rodríguez-Piñeiro AM, Cordero OJ, Rodríguez-Berrocal FJ, Ayude D, Rivas-Hervada And FJ, de la Cadena MP. Soluble CD26 levels and its association to epidemiologic parameters in a sample population. *Dis Markers* 2009; **27**: 311-316
110 **Cordero OJ**, De Chiara L, Lemos-González Y, Páez de la Cadena M, Rodríguez-Berrocal FJ. How the measurements of a few serum markers can be combined to enhance their clinical values in the management of cancer. *Anticancer Res* ; **28**: 2333-2341
111 **Cordero OJ**, De Chiara L, Páez de la Cadena M, Rodriguez-Berrocal,FJ . Validation of serum CD26 as a screening marker for colorectal cancer. *Clin Chem Lab Med* 2008; **46**: A23
112 **Trotta PP**, Balis ME. Characterization of adenosine deaminase from normal colon and colon tumors. Evidence for tumor-specific variants. *Biochemistry* 1978; **17**: 270-278
113 **ten Kate J**, Wijnen JT, van der Goes RG, Quadt R, Griffioen G, Bosman FT, Khan PM. Quantitative changes in adenosine deaminase isoenzymes in human colorectal adenocarcinomas. *Cancer Res* 1984; **44**: 4688-4692
114 **De Meester I**, Korom S, Van Damme J, Scharpé S. CD26, let it cut or cut it down. *Immunol Today* 1999; **20**: 367-375
115 **Šedo A**, Stremenová J, Bušek P, Duke-Cohan J. Dipeptidyl peptidase-IV and related molecules: markers of malignancy? *Expert Opin Med Diagn* 2008; **2**: 677-689
116 **Iwata S**, Morimoto C. CD26/dipeptidyl peptidase IV in context. The different roles of a multifunctional ectoenzyme in malignant transformation. *J Exp Med* 1999; **190:** 301-306
117 **Havre PA**, Abe M, Urasaki Y, Ohnuma K, Morimoto C, Dang NH. The role of CD26/dipeptidyl peptidase IV in cancer. *Front Biosci* 2008; **13**: 1634-1645
118 **Lambeir AM**, Durinx C, Scharpé S, De Meester I. Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV. *Crit Rev Clin Lab Sci* 2003; **40**: 209-294
119 **White N**, Burnstock G. P2 receptors and cancer. *Trends Pharmacol Sci* 2006; **27**: 211-217
120 **Pacheco R**, Martinez-Navio JM, Lejeune M, Climent N, Oliva H, Gatell JM, Gallart T, Mallol J, Lluis C, Franco R. CD26, adenosine deaminase, and adenosine receptors mediate costimulatory signals in the immunological synapse. *Proc Natl Acad Sci U S A* 2005; **102**: 9583-9588
121 **Herrera C**, Casadó V, Ciruela F, Schofield P, Mallol J, Lluis C, Franco R. Adenosine A2B receptors behave as an alternative anchoring protein for cell surface adenosine deaminase in lymphocytes and cultured cells. *Mol Pharmacol* 2001; **59**: 127-134
122 **Ginés S**, Mariño M, Mallol J, Canela EI, Morimoto C, Callebaut C, Hovanessian A, Casadó V, Lluis C, Franco R. Regulation of epithelial and lymphocyte cell adhesion by adenosine deaminase-CD26 interaction. *Biochem J* 2002; **361**: 203-209
123 **Cordero OJ**, Salgado FJ, Fernández-Alonso CM, Herrera C, Lluis C, Franco R, Nogueira M. Cytokines regulate membrane adenosine deaminase on human activated lymphocytes. *J Leukoc Biol* 2001; **70**: 920-930
124 **Hashikawa T**, Hooker SW, Maj JG, Knott-Craig CJ, Takedachi M, Murakami S, Thompson LF. Regulation of adenosine receptor engagement by ecto-adenosine deaminase. *FASEB J* 2004; **18**: 131-133
125 **Lukashev D**, Ohta A, Sitkovsky M. Hypoxia-dependent anti-inflammatory pathways in protection of cancerous tissues. *Cancer Metastasis Rev* 2007; **26**: 273-279
126 **Hoskin DW**, Mader JS, Furlong SJ, Conrad DM, Blay J. Inhibition of T cell and natural killer cell function by adenosine and its contribution to immune evasion by tumor cells (Review). *Int J Oncol* 2008; **32**: 527-535
127 **Gonzalez-Gronow M**, Kaczowka S, Gawdi G, Pizzo SV. Dipeptidyl peptidase IV (DPP IV/CD26) is a cell-surface plasminogen receptor. *Front Biosci* 2008; **13**: 1610-1618
128 **Chen WT**, Kelly T. Seprase complexes in cellular invasiveness. *Cancer Metastasis Rev* 2003; **22**: 259-269
129 **O'Brien P**, O'Connor BF. Seprase: an overview of an important matrix serine protease. *Biochim Biophys Acta* 2008; **1784**: 1130-1145

130 **Werb Z**. ECM and cell surface proteolysis: regulating cellular ecology. *Cell* 1997; **91**: 439-442

131 **Ghersi G**, Zhao Q, Salamone M, Yeh Y, Zucker S, Chen WT. The protease complex consisting of dipeptidyl peptidase IV and seprase plays a role in the migration and invasion of human endothelial cells in collagenous matrices. *Cancer Res* 2006; **66**: 4652-4661

132 **Piazza GA**, Callanan HM, Mowery J, Hixson DC. Evidence for a role of dipeptidyl peptidase IV in fibronectin-mediated interactions of hepatocytes with extracellular matrix. *Biochem J* 1989; **262**: 327-334

133 **Scanlan MJ,** Raj BK, Calvo B, Garin-Chesa P, Sanz-Moncasi MP, Healey JH, Old LJ, Rettig WJ. Molecular cloning of fibroblast activation protein alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. *Proc Natl Acad Sci U S A* 1994; **91**: 5657-5661

134 **Hartel S**, Gossrau R, Hanski C, Reutter W. Dipeptidyl peptidase (DPP) IV in rat organs. Comparison of immunohistochemistry and activity histochemistry. *Histochemistry* 1988; **89**: 151-161

135 **Boonacker E**, Van Noorden CJ. The multifunctional or moonlighting protein CD26/DPPIV. *Eur J Cell Biol* 2003; **82**: 53-73

136 **Ohnuma K**, Dang NH, Morimoto C. Revisiting an old acquaintance: CD26 and its molecular mechanisms in T cell function. *Trends Immunol* 2008; **29**: 295-301

137 **Gorrell MD**, Gysbers V, McCaughan GW. CD26: a multifunctional integral membrane and secreted protein of activated lymphocytes. *Scand J Immunol* 2001; **54**: 249-264

138 **Sedo A**, Busek P, Scholzová E, Malík R, Vlasicová K, Janácková S, Mares V. 'Dipeptidyl peptidase-IV activity and/or structure homologs' (DASH) in growth-modulated glioma cell lines. *Biol Chem* 2004; 385: 557-559

139 **Baggio LL**, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology* 2007; **132**: 2131-2157

140 **Drucker DJ**, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006; **368**: 1696-1705

141 **Baggio LL**, Drucker DJ. Therapeutic approaches to preserve islet mass in type 2 diabetes. *Annu Rev Med* 2006; **57**: 265-281

142 **Cordero OJ**, Salgado FJ, Nogueira M. On the origin of serum CD26 and its altered concentration in cancer patients. *Cancer Immunol Immunother* 2009; **58**: 1723-1747

143 **Davoodi J**, Kelly J, Gendron NH, MacKenzie AE. The Simpson-Golabi-Behmel syndrome causative glypican-3, binds to and inhibits the dipeptidyl peptidase activity of CD26. *Proteomics* 2007; **7**: 2300-2310

144 **Baumhoer D**, Tornillo L, Stadlmann S, Roncalli M, Diamantis EK, Terracciano LM. Glypican 3 expression in human nonneoplastic, preneoplastic, and neoplastic tissues: a tissue microarray analysis of 4,387 tissue samples. *Am J Clin Pathol* 2008; **129**: 899-906

145 **Houghton AN**, Albino AP, Cordon-Cardo C, Davis LJ, Eisinger M. Cell surface antigens of human melanocytes and melanoma. Expression of adenosine deaminase binding protein is extinguished with melanocyte transformation. *J Exp Med* 1988; **167**: 197-212

146 **Morrison ME**, Vijayasaradhi S, Engelstein D, Albino AP, Houghton AN. A marker for neoplastic progression of human melanocytes is a cell surface ectopeptidase. *J Exp Med* 1993; **177**: 1135-1143

147 **Albino AP**, Sozzi G, Nanus DM, Jhanwar SC, Houghton AN. Malignant transformation of human melanocytes: induction of a complete melanoma phenotype and genotype. *Oncogene* 1992; **7**: 2315-2321

148 **Wesley UV**, Albino AP, Tiwari S, Houghton AN. A role for dipeptidyl peptidase IV in suppressing the malignant phenotype of melanocytic cells. *J Exp Med* 1999; **190**: 311-322

149 **Wolf M**, Albrecht S, Märki C. Proteolytic processing of chemokines: implications in physiological and pathological conditions. *Int J Biochem Cell Biol* 2008; **40**: 1185-1198

150 **Preller V**, Gerber A, Wrenger S, Togni M, Marguet D, Tadje J, Lendeckel U, Röcken C, Faust J, Neubert K, Schraven B, Martin R, Ansorge S, Brocke S, Reinhold D. TGF-beta1-mediated control of central nervous system inflammation and autoimmunity through the inhibitory receptor CD26. *J Immunol* 2007; **178**: 4632-4640

151 **Coussens LM**, Werb Z. Inflammation and cancer. *Nature* 2002; **420**: 860-867

152 **Steinbrecher A**, Reinhold D, Quigley L, Gado A, Tresser N, Izikson L, Born I, Faust J, Neubert K, Martin R, Ansorge S, Brocke S. Targeting dipeptidyl peptidase IV (CD26) suppresses autoimmune encephalomyelitis and up-regulates TGF-beta 1 secretion in vivo. *J Immunol* 2001; **166**: 2041-2048

153 **Shingu K,** Helfritz A, Zielinska-Skowronek M, Meyer-Olson D, Jacobs R, Schmidt RE, Mentlein R, Pabst R, von Hörsten S. CD26 expression determines lung metastasis in mutant F344 rats: involvement of NK cell function and soluble CD26. *Cancer Immunol Immunother* 2003; **52**: 546-554

154 **Nagatsu I**, Nagatsu T, Yamamoto T. Hydrolysis of amino acid beta-naphthylamides by aminopeptidases in human parotid salva and human serum. *Experientia* 1968; **24**: 347-348

155 **Schrader WP**, Woodward FJ, Pollara B. Purification of an adenosine deaminase complexing protein from human plasma. J *Biol Chem* 1979; **254**: 11964-11968

156 **Iwaki-Egawa S**, Watanabe Y, Kikuya Y, Fujimoto Y. Dipeptidyl peptidase IV from human serum: purification, characterization, and N-terminal amino acid sequence. *J Biochem* 1998; **124**: 428-433

157 **Durinx C**, Lambeir AM, Bosmans E, Falmagne JB, Berghmans R, Haemers A, Scharpé S, De Meester I. Molecular characterization of dipeptidyl peptidase activity in serum: soluble CD26/dipeptidyl peptidase IV is responsible for the release of X-Pro dipeptides. *Eur J Biochem* 2000; **267**: 5608-5613

158 **Cuchacovich M**, Gatica H, Pizzo SV, Gonzalez-Gronow M. Characterization of human serum dipeptidyl peptidase IV (CD26) and analysis of its autoantibodies in patients with rheumatoid arthritis and other autoimmune diseases. *Clin Exp Rheumatol* 2001; **19**: 673-680

159 **Smith RE**, Talhouk JW, Brown EE, Edgar SE. The significance of hypersialylation of dipeptidyl peptidase IV (CD26) in the inhibition of its activity by Tat and other cationic peptides. CD26: a subverted adhesion molecule for HIV peptide binding. *AIDS Res Hum Retroviruses* 1998; **14**: 851-868

160 **Christopherson KW**, Hangoc G, Mantel CR, Broxmeyer HE. Modulation of hematopoietic stem cell homing and engraftment by CD26. *Science* 2004; **305**: 1000-1003

161 **Blanc G**, Font B, Eichenberger D, Moreau C, Ricard-Blum S, Hulmes DJ, Moali C. Insights into how CUB domains can exert specific functions while sharing a common fold: conserved and specific features of the CUB1 domain contribute to the molecular basis of procollagen C-proteinase enhancer-1 activity. *J Biol Chem* 2007; **282**: 16924-16933

162 **Wermter C**, Höwel M, Hintze V, Bombosch B, Aufenvenne K, Yiallouros I, Stöcker W. The protease domain of procollagen C-proteinase (BMP1) lacks substrate selectivity, which is conferred by non-proteolytic domains. *Biol Chem* 2007; **388**: 513-521

163 **Duke-Cohan JS**, Morimoto C, Rocker JA, Schlossman SF. A novel form of dipeptidylpeptidase IV found in human serum. Isolation, characterization, and comparison with T lymphocyte membrane dipeptidylpeptidase IV (CD26). *J Biol Chem* 1995; **270**: 14107-14114

164 **Duke-Cohan JS**, Morimoto C, Rocker JA, Schlossman SF. Serum high molecular weight dipeptidyl peptidase IV (CD26) is similar to a novel antigen DPPT-L released from activated T cells. *J Immunol* 1996; **156**: 1714-1721

165 **Friedrich D**, Hoffmann T, Bär J, Wermann M, Manhart S,

Heiser U, Demuth HU. Does human attractin have DP4 activity? *Biol Chem* 2007; **388**: 155-162
166 **Tang W**, Gunn TM, McLaughlin DF, Barsh GS, Schlossman SF, Duke-Cohan JS. Secreted and membrane attractin result from alternative splicing of the human ATRN gene. *Proc Natl Acad Sci U S A* 2000; **97**: 6025-6030
167 **Watanabe Y**, Ito K, Iwaki-Egawa S, Yamaguchi R, Fujimoto Y. Aminopeptidase N in sera of healthy subjects is a different N-terminal processed derivative from the one obtained from maternal serum. *Mol Genet Metab* 1998; **63**: 289-294
168 **Watanabe Y**, Iwaki-Egawa S, Mizukoshi H, Fujimoto Y. Identification of an alanine aminopeptidase in human maternal serum as a membrane-bound aminopeptidase N. *Biol Chem Hoppe Seyler* 1995; **376**: 397-400
169 **Cro L**, Morabito F, Zucal N, Fabris S, Lionetti M, Cutrona G, Rossi F, Gentile M, Ferrario A, Ferrarini M, Molica S, Neri A, Baldini L. CD26 expression in mature B-cell neoplasia: its possible role as a new prognostic marker in B-CLL. *Hematol Oncol* 2009; **27**: 140-147
170 **Bauvois B**, Djavaheri-Mergny M, Rouillard D, Dumont J, Wietzerbin J. Regulation of CD26/DPPIV gene expression by interferons and retinoic acid in tumor B cells. *Oncogene* 2000; **19**: 265-272
171 **Schade J,** Stephan M, Schmiedl A, Wagner L, Niestroj AJ, Demuth HU, Frerker N, Klemann C, Raber KA, Pabst R, von Hörsten S. Regulation of expression and function of dipeptidyl peptidase 4 (DP4), DP8/9, and DP10 in allergic responses of the lung in rats. *J Histochem Cytochem* 2008; **56**: 147-155
172 **Alfalah M**, Jacob R, Naim HY. Intestinal dipeptidyl peptidase IV is efficiently sorted to the apical membrane through the concerted action of N- and O-glycans as well as association with lipid microdomains. *J Biol Chem* 2002; **277**: 10683-10690
173 **Alfalah M**, Wetzel G, Fischer I, Busche R, Sterchi EE, Zimmer KP, Sallmann HP, Naim HY. A novel type of detergent-resistant membranes may contribute to an early protein sorting event in epithelial cells. *J Biol Chem* 2005; **280**: 42636-42643
174 **Danielsen EM**, Cowell GM, Poulsen SS. Biosynthesis of intestinal microvillar proteins. Role of the Golgi complex and microtubules. *Biochem J* 1983; **216**: 37-42
175 **Murphy G**, Murthy A, Khokha R. Clipping, shedding and RIPping keep immunity on cue. *Trends Immunol* 2008; **29**: 75-82
176 **Arribas J**, Coodly L, Vollmer P, Kishimoto TK, Rose-John S, Massagué J. Diverse cell surface protein ectodomains are shed by a system sensitive to metalloprotease inhibitors. *J Biol Chem* 1996; **271**: 11376-11382
177 **Lemberg MK**, Freeman M. Cutting proteins within lipid bilayers: rhomboid structure and mechanism. *Mol Cell* 2007; **28**: 930-940
178 **Grondin G**, Hooper NM, LeBel D. Specific localization of membrane dipeptidase and dipeptidyl peptidase IV in secretion granules of two different pancreatic islet cells. *J Histochem Cytochem* 1999; **47**: 489-498
179 **Poulsen MD**, Hansen GH, Dabelsteen E, Høyer PE, Norén O, Sjöström H. Dipeptidyl peptidase IV is sorted to the secretory granules in pancreatic islet A-cells. *J Histochem Cytochem* 1993; **41**: 81-88
180 **Macnair DC**, Kenny AJ. Proteins of the kidney microvillar membrane. The amphipathic form of dipeptidyl peptidase IV. *Biochem J* 1979; **179**: 379-395
181 **Théry C**, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol* 2002; **2**: 569-579
182 **Mignot G**, Roux S, Thery C, Ségura E, Zitvogel L. Prospects for exosomes in immunotherapy of cancer. *J Cell Mol Med* 2006; **10**: 376-388
183 **Schorey JS**, Bhatnagar S. Exosome function: from tumor immunology to pathogen biology. *Traffic* 2008; **9**: 871-881
184 **Gatti JL**, Métayer S, Belghazi M, Dacheux F, Dacheux JL. Identification, proteomic profiling, and origin of ram epididymal fluid exosome-like vesicles. *Biol Reprod* 2005; **72**: 1452-1465
185 **Mallegol J**, van Niel G, Heyman M. Phenotypic and functional characterization of intestinal epithelial exosomes. *Blood Cells Mol Dis* 2005; **35**: 11-16
186 **Ogawa Y**, Kanai-Azuma M, Akimoto Y, Kawakami H, Yanoshita R. Exosome-like vesicles with dipeptidyl peptidase IV in human saliva. *Biol Pharm Bull* 2008; **31**: 1059-1062
187 **Hino M**, Nagatsu T, Kakumu S, Okuyama S, Yoshii Y, Nagatsu I. Glycylprolyl beta-naphthylamidase activity in human serum. *Clin Chim Acta* 1975; **62**: 5-11
188 **Ten Kate J**, Wijnen JT, Boldewijn J, Khan PM, Bosman FT. Immunohistochemical localization of adenosine deaminase complexing protein in intestinal mucosa and in colorectal adenocarcinoma as a marker for tumour cell heterogeneity. *Histochem J* 1985; **17:** 23-31
189 **Kojima J**, Kanatani M, Kato M, Tojoh F, Nakamura N. Serum glycylproline dipeptidyl aminopeptidase activity in human hepatic cancer. *Clin Chim Acta* 1979; **93**: 181-187
190 **Perner F**, Gyuris T, Rákóczy G, Sárváry E, Görög D, Szalay F, Kunos I, Szönyi L, Péterfy M, Takács L. Dipeptidyl peptidase activity of CD26 in serum and urine as a marker of cholestasis: experimental and clinical evidence. *J Lab Clin Med* 1999; **134**: 56-67
191 **Bartles JR**, Zhang LQ, Verheyen EM, Hospodar KS, Nehme CL, Fayos BE. Decreases in the relative concentrations of specific hepatocyte plasma membrane proteins during liver regeneration: down-regulation or dilution? *Dev Biol* 1991; 143: 258-270
192 **Bartles JR**, Rao MS, Zhang LQ, Fayos BE, Nehme CL, Reddy JK. Expression and compartmentalization of integral plasma membrane proteins by hepatocytes and their progenitors in the rat pancreas. *J Cell Sci* 1991; **98** (Pt 1): 45-54
193 **Lakatos PL**, Firneisz G, Rákóczy G, Selmeci L, Szalay F. Elevated serum dipeptidyl peptidase IV (CD26, EC 3.4.14.5) activity in patients with primary biliary cirrhosis. *J Hepatol* 1999; **30**: 740
194 **Sahara N**, Fukasawa K, Harada M, Suzuki K. Immunohistochemical localization of dipeptidyl peptidase IV in rat digestive organs. *Acta Histochem Cytochem* 1983; **16**: 494-501
195 **Andrieu T**, Thibault V, Malet I, Laporte J, Bauvois B, Agut H, Cahour A. Similar increased serum dipeptidyl peptidase IV activity in chronic hepatitis C and other viral infections. *J Clin Virol* 2003; **27**: 59-68
196 **McCaughan GW**, Gorrell MD, Bishop GA, Abbott CA, Shackel NA, McGuinness PH, Levy MT, Sharland AF, Bowen DG, Yu D, Slaitini L, Church WB, Napoli J. Molecular pathogenesis of liver disease: an approach to hepatic inflammation, cirrhosis and liver transplant tolerance. *Immunol Rev* 2000; **174**: 172-191
197 **Kasahara Y**, Leroux-Roels G, Nakamura R, Chisari F. Glycylprolyl-diaminopeptidase in human leukocytes: selective occurrence in T lymphocytes and influence on the total serum enzyme activity. *Clin Chim Acta* 1984; **139**: 295-302
198 **Kahne T**, Lendeckel U, Wrenger S, Neubert K, Ansorge S, Reinhold D. Dipeptidyl peptidase IV: a cell surface peptidase involved in regulating T cell growth (review). *Int J Mol Med* 1999; **4**: 3-15
199 **Ward PE**. Immunoelectrophoretic analysis of vascular, membrane-bound angiotensin I converting enzyme, aminopeptidase M, and dipeptidyl(amino)peptidase IV. *Biochem Pharmacol* 1984; **33**: 3183-3193
200 **Mentzel S**, Dijkman HB, Van Son JP, Koene RA, Assmann KJ. Organ distribution of aminopeptidase A and dipeptidyl peptidase IV in normal mice. *J Histochem Cytochem* 1996; **44**: 445-461

201 **Salgado FJ**, Lojo J, Alonso-Lebrero JL, Lluis C, Franco R, Cordero OJ, Nogueira M. A role for interleukin-12 in the regulation of T cell plasma membrane compartmentation. *J Biol Chem* 2003; 278: 24849-24857
202 **Cordero OJ**, Salgado FJ, Viñuela JE, Nogueira M. Interleukin-12 enhances CD26 expression and dipeptidyl peptidase IV function on human activated lymphocytes. *Immunobiology* 1997; **197**: 522-533
203 **Uematsu T**, Urade M, Yamaoka M. Decreased expression and release of dipeptidyl peptidase IV (CD26) in cultured peripheral blood T lymphocytes of oral cancer patients. *J Oral Pathol Med* 1998; **27**: 106-110
204 **Uematsu T**, Tanaka H, Yamaoka M, Furusawa K. Effects of oral squamous cell carcinoma-derived TGF-beta1 on CD26/DPPIV expression in T cells. *Anticancer Res* 2004; **24**: 619-624
205 **Teague TK**, Hildeman D, Kedl RM, Mitchell T, Rees W, Schaefer BC, Bender J, Kappler J, Marrack P. Activation changes the spectrum but not the diversity of genes expressed by T cells. *Proc Natl Acad Sci U S A* 1999; **96**: 12691-12696
206 **Rogge L**, Bianchi E, Biffi M, Bono E, Chang SY, Alexander H, Santini C, Ferrari G, Sinigaglia L, Seiler M, Neeb M, Mous J, Sinigaglia F, Certa U. Transcript imaging of the development of human T helper cells using oligonucleotide arrays. *Nat Genet* 2000; **25**: 96-101
207 **Lojda Z**. Studies on dipeptidyl(amino)peptidase IV (glycyl-proline naphthylamidase). II. Blood vessels. *Histochemistry* 1979; **59**: 153-166
208 **van der Velden VH**, Wierenga-Wolf AF, Adriaansen-Soeting PW, Overbeek SE, Möller GM, Hoogsteden HC, Versnel MA. Expression of aminopeptidase N and dipeptidyl peptidase IV in the healthy and asthmatic bronchus. *Clin Exp Allergy* 1998; **28**: 110-120
209 **Gossrau R**. [Peptidases II. Localization of dipeptidylpeptidase IV (DPP IV). Histochemical and biochemical study]. *Histochemistry* 1979; **60**: 231-248
210 **Pala L**, Mannucci E, Pezzatini A, Ciani S, Sardi J, Raimondi L, Ognibene A, Cappadona A, Vannelli BG, Rotella CM. Dipeptidyl peptidase-IV expression and activity in human glomerular endothelial cells. *Biochem Biophys Res Commun* 2003; **310**: 28-31
211 **Cuchacovich M**, Gatica H, Vial P, Yovanovich J, Pizzo SV, Gonzalez-Gronow M. Streptokinase promotes development of dipeptidyl peptidase IV (CD26) autoantibodies after fibrinolytic therapy in myocardial infarction patients. *Clin Diagn Lab Immunol* 2002; **9**: 1253-1259
212 **Cordon-Cardo C**, Prives C. At the crossroads of inflammation and tumorigenesis. *J Exp Med* 1999; **190**: 1367-1370
213 **Dvorak HF**. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med* 1986; **315**: 1650-1659
214 **Busso N**, Wagtmann N, Herling C, Chobaz-Péclat V, Bischof-Delaloye A, So A, Grouzmann E. Circulating CD26 is negatively associated with inflammation in human and experimental arthritis. *Am J Pathol* 2005; **166**: 433-442
215 **Narducci MG**, Scala E, Bresin A, Caprini E, Picchio MC, Remotti D, Ragone G, Nasorri F, Frontani M, Arcelli D, Volinia S, Lombardo GA, Baliva G, Napolitano M, Russo G. Skin homing of Sézary cells involves SDF-1-CXCR4 signaling and down-regulation of CD26/dipeptidylpeptidase IV. *Blood* 2006; **107**: 1108-1115
216 **Byrd JB**, Touzin K, Sile S, Gainer JV, Yu C, Nadeau J, Adam A, Brown NJ. Dipeptidyl peptidase IV in angiotensin-converting enzyme inhibitor associated angioedema. *Hypertension* 2008; **51**: 141-147
217 **Fryer RM**, Segreti J, Banfor PN, Widomski DL, Backes BJ, Lin CW, Ballaron SJ, Cox BF, Trevillyan JM, Reinhart GA, von Geldern TW. Effect of bradykinin metabolism inhibitors on evoked hypotension in rats: rank efficacy of enzymes associated with bradykinin-mediated angioedema. *Br J Pharmacol* 2008; 153: 947-955
218 **Pesquero JB**, Jubilut GN, Lindsey CJ, Paiva AC. Bradykinin metabolism pathway in the rat pulmonary circulation. *J Hypertens* 1992; **10**: 1471-1478
219 **Mentlein R**, Roos T. Proteases involved in the metabolism of angiotensin II, bradykinin, calcitonin gene-related peptide (CGRP), and neuropeptide Y by vascular smooth muscle cells. *Peptides* 1996; **17**: 709-720
220 **Brandt I**, Lambeir AM, Ketelslegers JM, Vanderheyden M, Scharpé S, De Meester I. Dipeptidyl-peptidase IV converts intact B-type natriuretic peptide into its des-SerPro form. *Clin Chem* 2006; **52**: 82-87
221 **Vanderheyden M**. Clinical importance of BNP truncation by DPPIV. *Clin Chem Lab Med* 2008; **46**: A18
222 **Abe K**, Tilan JU, Zukowska Z. NPY and NPY receptors in vascular remodeling. *Curr Top Med Chem* 2007; **7**: 1704-1709
223 **Kuo LE**, Kitlinska JB, Tilan JU, Li L, Baker SB, Johnson MD, Lee EW, Burnett MS, Fricke ST, Kvetnansky R, Herzog H, Zukowska Z. Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. *Nat Med* 2007; **13**: 803-811
224 **Kitlinska J**, Abe K, Kuo L, Pons J, Yu M, Li L, Tilan J, Everhart L, Lee EW, Zukowska Z, Toretsky JA. Differential effects of neuropeptide Y on the growth and vascularization of neural crest-derived tumors. *Cancer Res* 2005; **65**: 1719-1728
225 **Dinjens WN**, Ten Kate J, Kirch JA, Tanke HJ, Van der Linden EP, Van den Ingh HF, Van Steenbrugge GJ, Meera Khan P, Bosman FT. Adenosine deaminase complexing protein (ADCP) expression and metastatic potential in prostatic adenocarcinomas. *J Pathol* 1990; **160**: 195-201
226 **Martín M**, Huguet J, Centelles JJ, Franco R. Expression of ecto-adenosine deaminase and CD26 in human T cells triggered by the TCR-CD3 complex. Possible role of adenosine deaminase as costimulatory molecule. *J Immunol* 1995; **155**: 4630-4643
227 **Sedo A**, Krepela E, Kasafírek E. Dipeptidyl peptidase IV, prolyl endopeptidase and cathepsin B activities in primary human lung tumors and lung parenchyma. *J Cancer Res Clin Oncol* 1991; **117**: 249-253
228 **Ayude D**, Páez de la Cadena M, Cordero OJ, Nogueira M, Ayude J, Fernández-Briera A, Rodríguez-Berrocal FJ. Clinical interest of the combined use of serum CD26 and alpha-L-fucosidase in the early diagnosis of colorectal cancer. *Dis Markers* 2003 ; **19**: 267-272
229 **de la Haba-Rodríguez J**, Macho A, Calzado MA, Blázquez MV, Gómez MA, Muñoz EE, Aranda E. Soluble dipeptidyl peptidase IV (CD-26) in serum of patients with colorectal carcinoma. *Neoplasma* 2002; **49**: 307-311
230 **Kobayashi H**, Hosono O, Mimori T, Kawasaki H, Dang NH, Tanaka H, Morimoto C. Reduction of serum soluble CD26/dipeptidyl peptidase IV enzyme activity and its correlation with disease activity in systemic lupus erythematosus. *J Rheumatol* 2002; **29**: 1858-1866
231 **Schönermarck U**, Csernok E, Trabandt A, Hansen H, Gross WL. Circulating cytokines and soluble CD23, CD26 and CD30 in ANCA-associated vasculitides. *Clin Exp Rheumatol* 2000; **18**: 457-463
232 **Yang SS**, Fu LS, Chang CS, Yeh HZ, Chen GH, Kao JH. Changes of soluble CD26 and CD30 levels correlate with response to interferon plus ribavirin therapy in patients with chronic hepatitis C. *J Gastroenterol Hepatol* 2006; **21**: 1789-1793
233 **Ajdary S**, Riazi-Rad F, Jafari-Shakib R, Mohebbali M. Soluble CD26/CD30 levels in visceral leishmaniasis: markers of disease activity. *Clin Exp Immunol* 2006; **145**: 44-47
234 **Ajdary S**, Jafari-Shakib R, Riazi-Rad F, Khamesipour A. Soluble CD26 and CD30 levels in patients with anthroponotic cutaneous leishmaniasis. *J Infect* 2007; **55**: 75-78
235 **Kojima K**, Mihara R, Sakai T, Togari A, Matsui T, Shinpo K, Fujita K, Fukasawa K, Harada M, Nagatsu T. Serum

activities of dipeptidyl-aminopeptidase II and dipeptidyl-aminopeptidase IV in tumor-bearing animals and in cancer patients. *Biochem Med Metab Biol* 1987; **37**: 35-41

236 **Rose M**, Hildebrandt M, Fliege H, Seibold S, Mönnikes H, Klapp BF. T-cell immune parameters and depression in patients with Crohn's disease. *J Clin Gastroenterol* 2002; **34**: 40-48

237 **Van Der Velden VH**, Naber BA, Van Hal PT, Overbeek SE, Hoogsteden HC, Versnel MA. Peptidase activities in serum and bronchoalveolar lavage fluid from allergic asthmatics--comparison with healthy non-smokers and smokers and effects of inhaled glucocorticoids. *Clin Exp Allergy* 1999; **29**: 813-823

238 **Jarmołowska B**, Bielikowicz K, Iwan M, Sidor K, Kostyra E, Kaczmarski M. Serum activity of dipeptidyl peptidase IV (DPPIV; EC 3.4.14.5) in breast-fed infants with symptoms of allergy. *Peptides* 2007; **28**: 678-682

239 **Detel D**, Persić M, Varljen J. Serum and intestinal dipeptidyl peptidase IV (DPP IV/CD26) activity in children with celiac disease. *J Pediatr Gastroenterol Nutr* 2007; **45**: 65-70

240 **Maes M**, Lin A, Bonaccorso S, Vandoolaeghe E, Song C, Goossens F, De Meester I, Degroote J, Neels H, Scharpé S, Janca A. Lower activity of serum peptidases in abstinent alcohol-dependent patients. *Alcohol* 1999; **17**: 1-6

241 **Allison JE**, Tekawa IS, Ransom LJ, Adrain AL. A comparison of fecal occult-blood tests for colorectal-cancer screening. *N Engl J Med* 1996; **334**: 155-159

242 **Zisman TL**, Rubin DT. Colorectal cancer and dysplasia in inflammatory bowel disease. *World J Gastroenterol* 2008; **14**: 2662-2669

243 **Rennert G**, Rennert HS, Miron E, Peterburg Y. Population colorectal cancer screening with fecal occult blood test. *Cancer Epidemiol Biomarkers Prev* 2001; **10**: 1165-1168

244 **Hazazi R**, Rozen P, Leshno M, Levi Z, Samuel Z, Waked A, Vilkin A, Maoz E, Birkenfeld S, Niv Y. Can patients at high risk for significant colorectal neoplasms and having normal quantitative faecal occult blood test postpone elective colonoscopy? *Aliment Pharmacol Ther* 2010; **31**: 523-533

245 **ten Kate J**, van den Ingh HF, Khan PM, Bosman FT. Adenosine deaminase complexing protein (ADCP) immunoreactivity in colorectal adenocarcinoma. *Int J Cancer* 1986; **37**: 479-485

246 **Shimwell NJ**, Wei W, Wilson S, Wakelam MJ, Ismail T, Iqbal T, Johnson PJ, Martin A, Ward DG. Assessment of novel combinations of biomarkers for the detection of colorectal cancer. *Cancer Biomark* 2010; **7**: 123-132

247 **Lemos-González Y**, Rodríguez-Berrocal FJ, Cordero OJ, Gómez C, Páez de la Cadena M. Alteration of the serum levels of the epidermal growth factor receptor and its ligands in patients with non-small cell lung cancer and head and neck carcinoma. *Br J Cancer* 2007; **96**: 1569-1578

248 **Ray S**, Britschgi M, Herbert C, Takeda-Uchimura Y, Boxer A, Blennow K, Friedman LF, Galasko DR, Jutel M, Karydas A, Kaye JA, Leszek J, Miller BL, Minthon L, Quinn JF, Rabinovici GD, Robinson WH, Sabbagh MN, So YT, Sparks DL, Tabaton M, Tinklenberg J, Yesavage JA, Tibshirani R, Wyss-Coray T. Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. *Nat Med* 2007; **13**: 1359-1362

249 **Borgia JA**, Basu S, Faber LP, Kim AW, Coon JS, Kaiser-Walters KA, Fhied C, Thomas S, Rouhi O, Warren WH, Bonomi P, Liptay MJ. Establishment of a multi-analyte serum biomarker panel to identify lymph node metastases in non-small cell lung cancer. *J Thorac Oncol* 2009; **4**: 338-347

250 **Croswell JM**, Kramer BS, Kreimer AR, Prorok PC, Xu JL, Baker SG, Fagerstrom R, Riley TL, Clapp JD, Berg CD, Gohagan JK, Andriole GL, Chia D, Church TR, Crawford ED, Fouad MN, Gelmann EP, Lamerato L, Reding DJ, Schoen RE. Cumulative incidence of false-positive results in repeated, multimodal cancer screening. *Ann Fam Med* 2009; **7**: 212-222

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