Tumor Markers and their correct use in neoplastic disease

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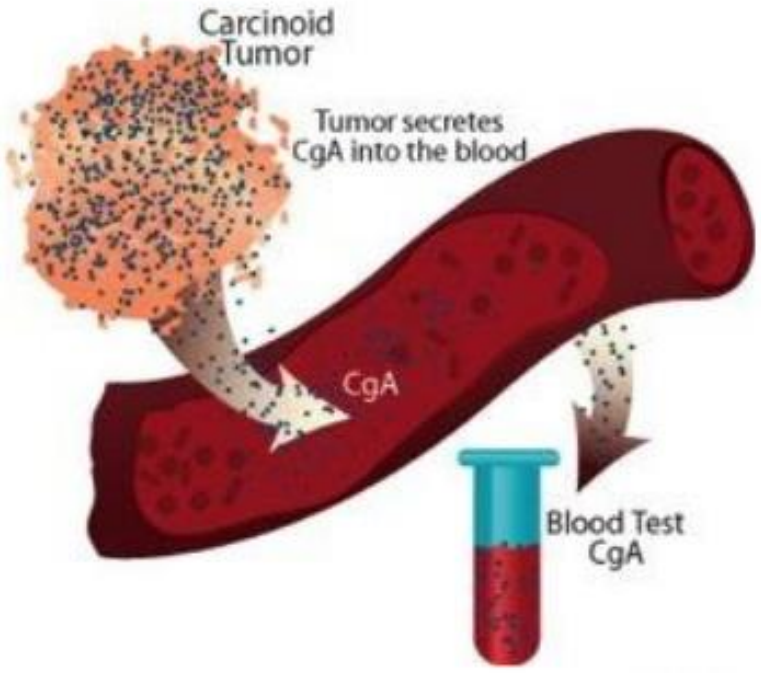
Tumor Markers

- Tumor Markers are molecules (generally glycoproteins), which levels may be elevated in the presence of a cancer, either as a reaction of the host to the tumor or as a product of the tumor itself.
- Useful for diagnosis, prognosis, treatment monitoring and relapse surveillance in different types of cancer.
- Currently, there are over 20 well known parameters that are widely considered as TM:
  - PSA —Prostate—; CA 15.3 —Breast—; CA 125 y HE4 —Ovarian—; CEA y CA 19.9 — Colorectal, Gastric and Pancreatic—: NSE y ProGRP —Lung—; etc.
- They are not specific to any type of cancer:
  - The differences between benign and malignant diseases are quantitative.



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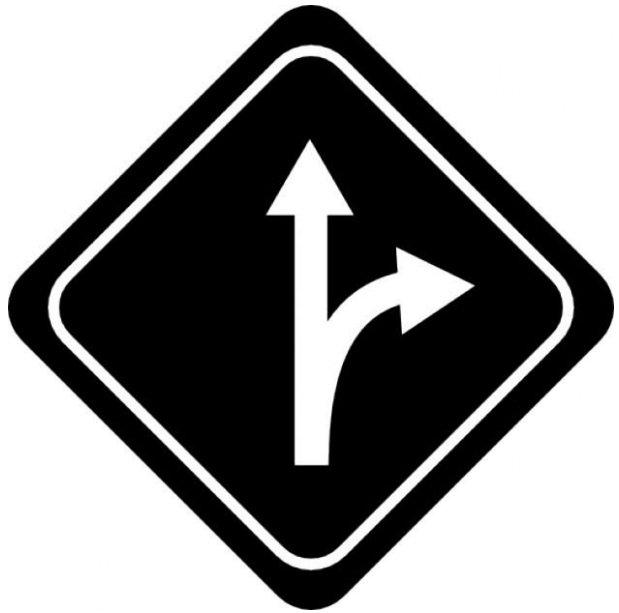
False Positives

- However, there is a variety of factors that can affect the accuracy of TM as they increase their levels without the presence of malignancy, resulting in false positives:
  - Benign Diseases
  - Drug Intake
  - Lifestyle
  - Technical Interferences
- The Spanish Society of Clinical Biochemistry and Molecular Pathology (Cancer Biomarkers Commission) established the Barcelona Criteria to:
  - Evaluate correctly the results and reduce the number of false positives.
  - Reduce the number of complementary scans.
  - Decrease waiting lists.
  - Improve quality of care.



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Barcelona Criteria

1. Evaluate TM Serum concentrations.
2. Discard benign pathology by the exclusion of main source False Positive results.
3. Follow-up in case of moderate TM results (Grey Zone/Undetermined).
4. Void Technical interferences.

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First Criteria: Tumor Markers Serum concentrations

- Serum levels of most TM, observed in the absence of neoplasia, are usually low or moderate.
- The higher is the concentration of a TM detected in a patient, the higher is the probability of being a malignant tumor:
  - For example, NSE levels below 40 ng/mL can be detected in numerous benign diseases, but levels above that value indicate a high probability of cancer or hemolysis.
  - The same occurs with concentrations of CA 125 and CA 19.9 higher than 1000 U/mL, or CEA values higher than 25 ng/mL, indicating higher than 95% probability of a malignant tumor.

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Second Criteria: Discard benign pathologies

- In case of an increase in a TM, it is necessary to discard the presence of certain benign pathologies that may be the cause.
- Most of the TM are catabolized at the liver level and excreted via the kidney.
- The alterations of these organs cause less catabolism and/or elimination and indirectly cause their accumulation, with values higher than the range considered as within normality:
  - Hepatopathies (AFP, CEA, CYFRA and HE4, among others).
  - Renal Insufficiency (CEA, CYFRA, NSE or ProGRP, among others).
- In other occasions, before certain comorbidities the value of a TM should be totally discarded:
  - Renal Insufficiency (HE4, SCC or S100).
  - Pemphigus or Psoriasis (SCC).
  - Benign Prostatic Hypertrophy or Prostatitis (PSA).
- Finally, there are also mixed causes of false positives such as CA 19.9 in Chronic Liver Disease with Jaundice, or due to stimulation by various drugs, as in the case of CA 72.4.

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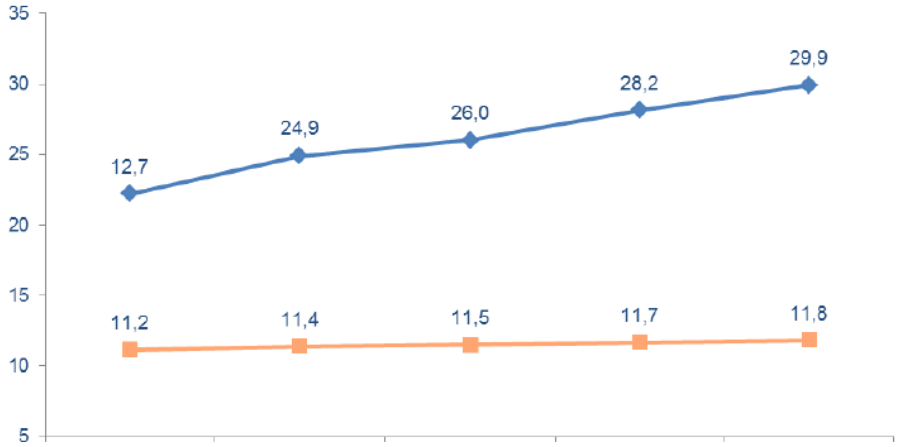
Third Criteria: Follow-up if moderate Tumor Markers results

- In some cases, the finding of elevated levels of any TM in isolation is of limited value.
- When there are doubts about a result, two serial determinations (evolutive control) should be performed with a longer time interval than their plasma half-life (15-20 days for the most of TM):
  - If the TM has a continuous increase (> 50%) over time (above the upper level of the reference range), it can be said that with a high probability it is of tumor origin, since they reflect the growth of the tumor.
  - In contrast, if serum levels do not change or tend to decline, the origin will have to be sought in another non-neoplastic pathology.



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Fourth Criteria: Void Technical interferences

- It is also important to discard values from samples that have experienced technical problems:
  - Lack of antibody specificity, cross-reactions with other molecules or the presence of heterophile antibodies:
  - Hemolyzed samples (invalidate NSE values).
- In addition, the results of a TM obtained by a commercial method should not be considered to be similar with others, and there may be notable discrepancies if used individually, being reduced as part of a panel by not only evaluating arbitrary values (cutoffs).

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Help Comments

- All MBDAA tests also provide valuable information for physicians by including guidance comments related to the results of TM levels as well as the existence of different comorbidities.
- Comments are chosen from a database with more than 600 pre-established comments.
- They take into account gender, age, different comorbidities, TM levels as well as the smoking habits of patients to explain the incidence of these factors in the final results (as the main causes of false positives).

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Statistical measurements in diagnostic tests

- Unfortunately, the use of TM in the routine also presents other problems:
  - Low sensitivity in early stages.
  - No specific TM for each malignant tumor.
- However, the combination of 2 or more TM (in the form of TM panels) yields better results, especially in advanced stages.
- In this way, the combination of several TM —as well as the inclusion of information from the patient's clinical history in the calculations— using complex algorithms with multiple variables results in higher sensitivity and specificity: this is what Bioprognos has called as MBDAA (Multiple Biomarkers Disease Activity Algorithms).

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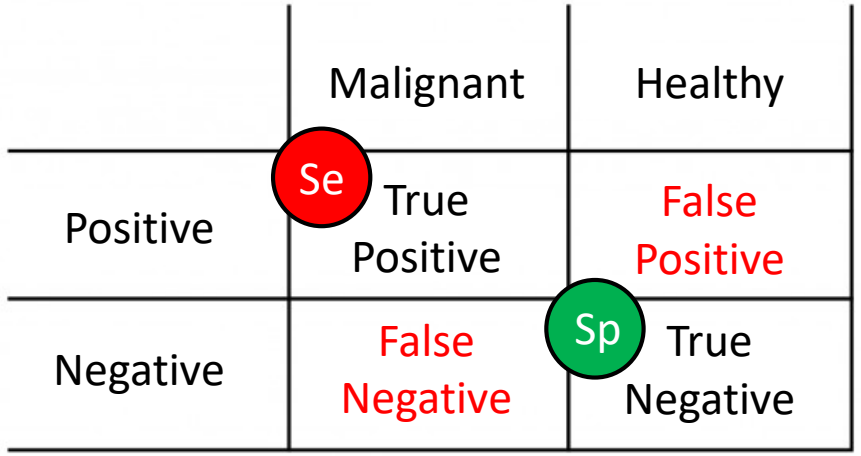
Statistical measurements in diagnostic tests: Sensitivity, Specificity, PPV and NPV

- Sensitivity (Se):
  - Percentage of real positives that are correctly identified.
  - Ability to test to get right with sick individuals (it measures the number of malignant tumors that are correctly identified as cancer).
- Specificity (Sp):
  - Proportion of true negatives that are correctly classified.
  - Ability of a test to get right with healthy individuals (measures the number of non-malignant tumors that are correctly identified as benign).
- Positive and negative predictive values :
  - The Positive Predictive Value (PPV) is the number of true positives correctly identified on the total of real positives. A test with many false positives (FP) will have a low VPP.
  - On the other hand, the Negative Predictive Value (NPV) is the number of true negatives (TN) correctly identified on the total of actual negatives. A high NPV value means that very few true positives (TP) were incorrectly identified as negative.



Se = TP / (TP + FN)
Sp = TN / (TN + FP)
PPV = TP / (TP + FP)
NPV = TN / (TN + FN)
IY = Se + Sp -1

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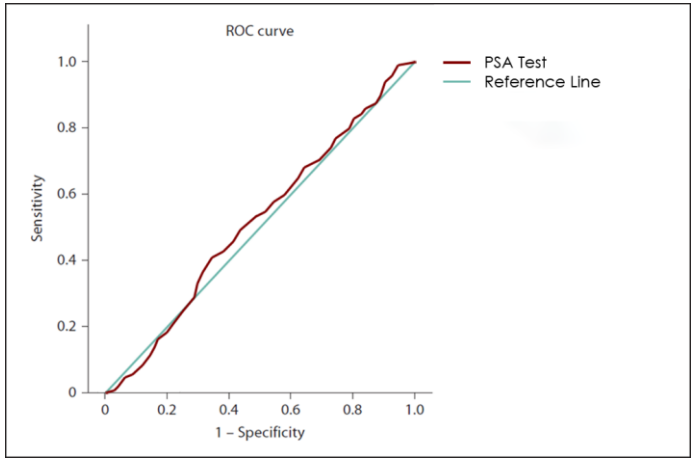
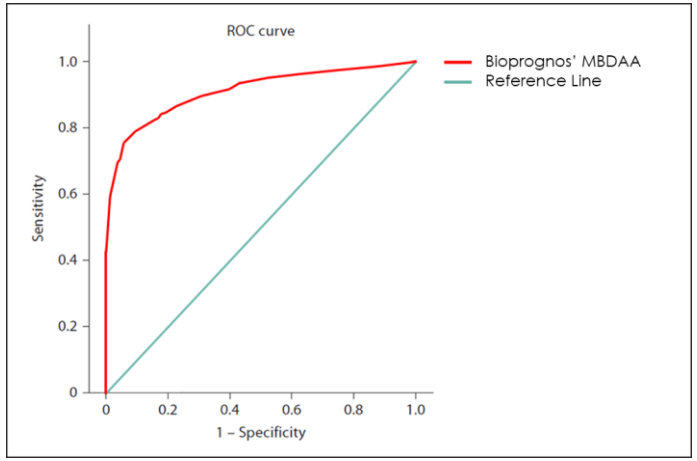
Statistical measurements in diagnostic tests: Receiving Operator Curves

- All of these different parameters can be represented together in a graph known as Receiving Operator Curve (ROC), where better results are shown with curves that tend to approach the upper left corner of the image (100% Sensitivity and 100% Specificity).



ROC Curve for the PSA Test for Prostate Cancer



ROC Curve for the MBDAA Test for Prostate Cancer

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MBDAA Tests to help in Cancer Diagnosis

- For years, imaging diagnosis and tissue biopsies have been at the forefront of cancer treatment, providing important insights about it, especially when it comes to detection.
- But these procedures are inherently limited:
  - The added risk and stress associated with biopsy can be a difficult situation for patients.
  - On the other hand, for those with advanced or inaccessible cancers, biopsy and imaging may not be an option.
  - In addition, the CT Scans may be less invasive but do not provide critical molecular information and all of these procedures add significant costs during the course of treatment.
- BIOPROGNOS has developed its own MBDAA Tests in order to create the first innovative, non-invasive, efficient and low cost line for diagnosis.

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MBDAA Tests to help in Cancer Diagnosis

- The MBDAA Tests are designed for the qualitative detection of different TM associated with neoplasms.
- The MBDAA Tests are especially indicated to examine men and women older than 40 years who have a typical average risk for lung, ovary or prostate cancer:
  - Any positive results should be followed and verified by other diagnostic procedures.
  - On the other hand, in the event of a negative result, patients should continue to participate in a screening program in an interval and with an appropriate method for each patient.
- They allow to assign additional diagnosis procedures and biopsies for those patients most likely to have cancer.
- They provide valuable information to patients with advanced cancers who can not be candidates for other invasive procedures.

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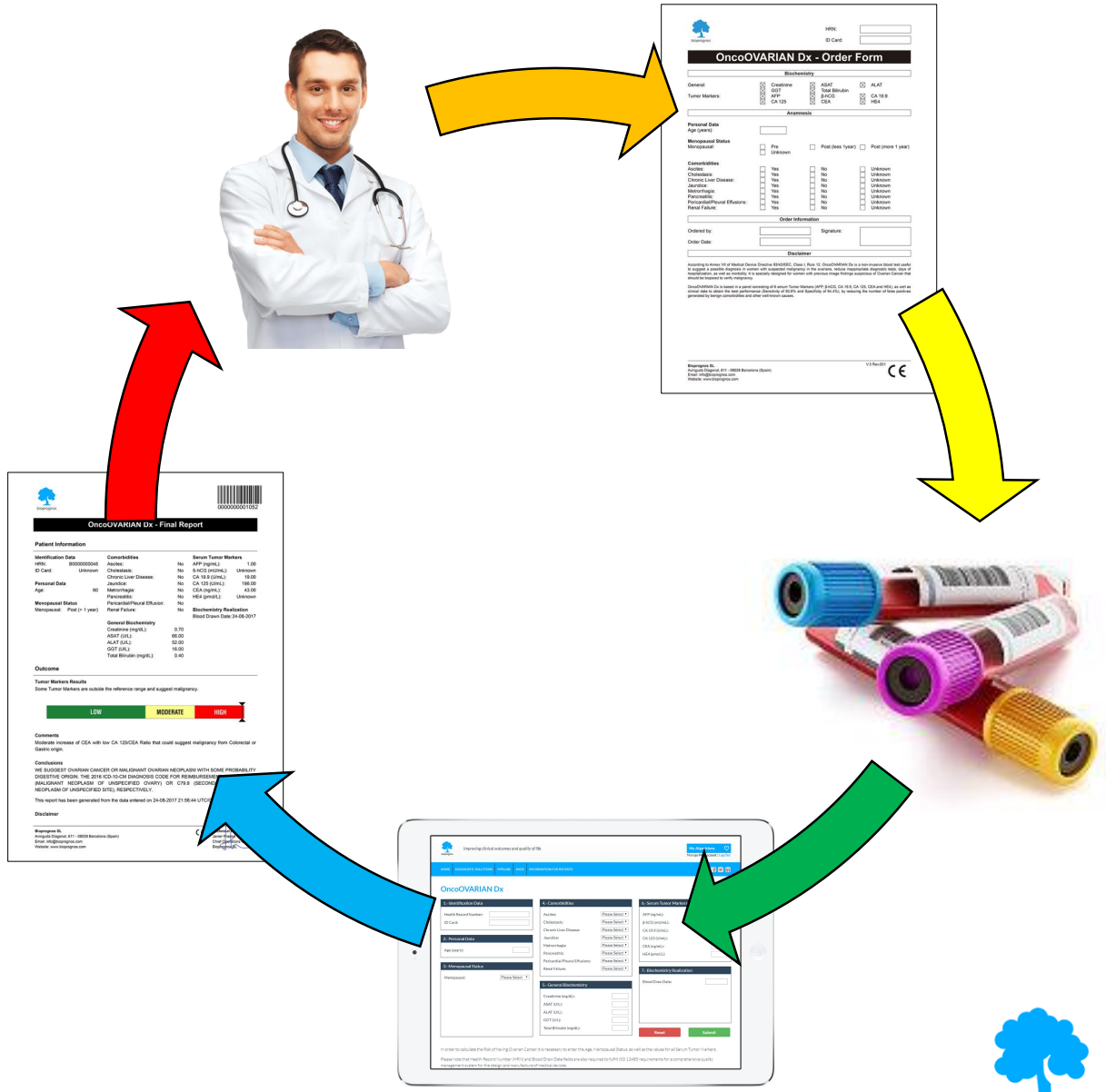
MBDAA Tests to help in Cancer Diagnosis

- The MBDAA Tests work in 5 simple steps:
    1. Doctor requests the tests through the Order Form sheets.
    2. Blood or urine drawn are performed in the laboratory (local partner of Bioprognos).
    3. Analytical information is entered in our server and the report is obtained.
    4. Report is sent to the doctor.
    5. Doctor uses it as a "Clinical Decision Support System" (CDSS) for the final diagnosis.



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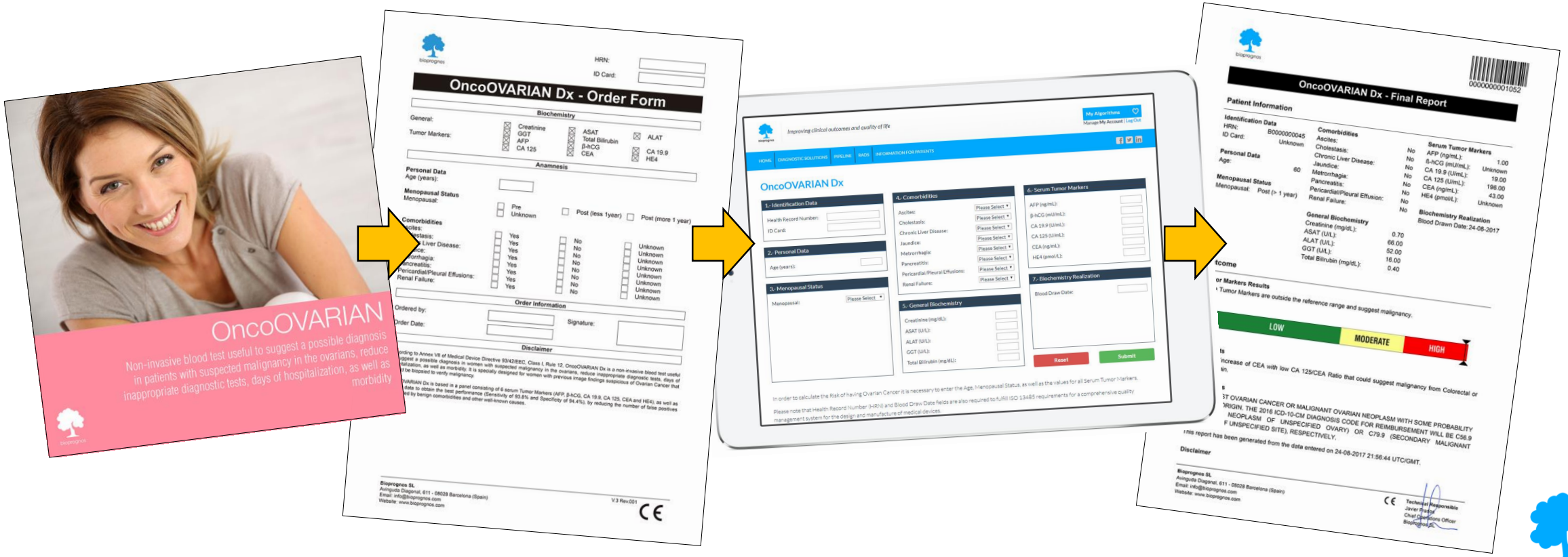
MBDAA Tests to help in Cancer Diagnosis

- Workflow:
  - Bioprognos provides all the necessary materials (brochures, order forms, online system for calculating risk as well as the final report in PDF), while blood and urine tests are the only step to be carried out locally.



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MBDAA Tests to help in Cancer Diagnosis

- BIOPROGNOS has already developed several non-invasive MBDAA Tests for the diagnosis of cancer based on different panels of TM:
  - MBDAA Test for Lung Cancer
  - MBDAA Test for Ovarian Cancer
  - MBDAA Test for Prostate Cancer
  - MBDAA Test for Cancer Unknown Primary
- All BIOPROGNOS MBDAA Tests are already certified with CE Declaration of Conformity, Class I, Rule 12, According to Annex VII of Medical Device Directive 93/42/EEC.

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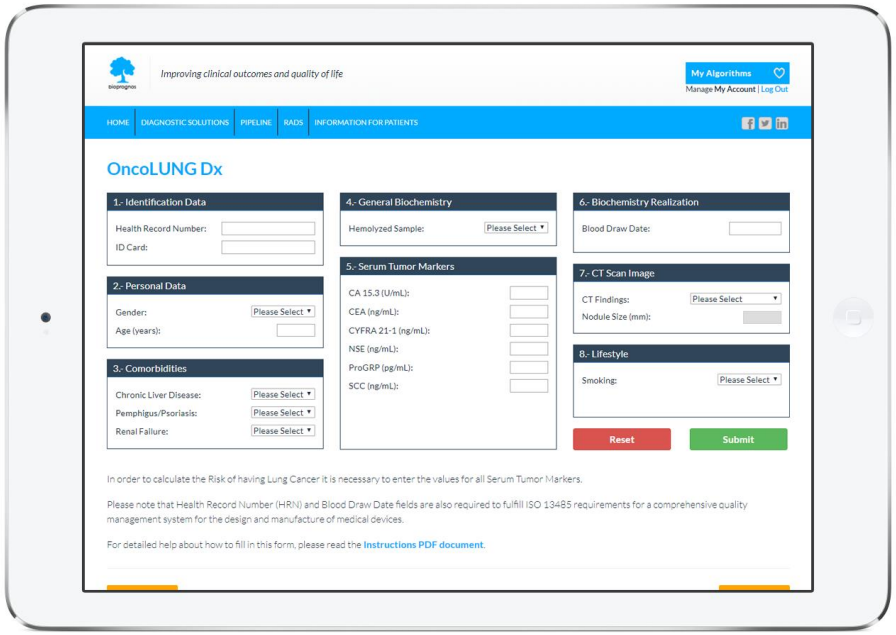
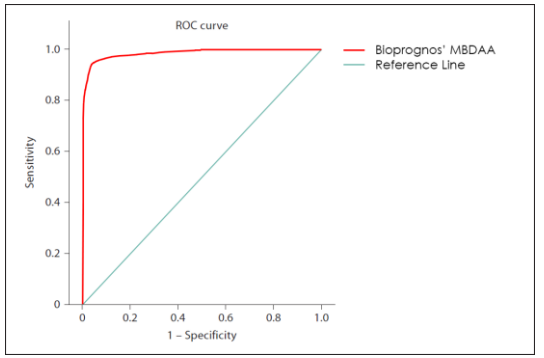
MBDAA Tests to help in Cancer Diagnosis: Lung Cancer

- Panel consisting of 6 TM:
  - CA 15.3, CEA, CYFRA, NSE, ProGRP and SCC.
- 4 comorbidities associated with false positives in the TM used in this panel:
  - Chronic Liver Disease, Pemphigus, Psoriasis and Renal Failure.
- Lifestyle associated with false positives in the TM used in this panel:
  - Smoking Habits.
- Addressed to:
  - Smokers older than 55 years.
  - Patients with previous image findings suspicious of Lung Cancer that should be biopsied to verify malignancy.
- Developed from research:
  - Molina, R., Marrades R. M., Auge J. M., Escudero J. M., Vinolas N., Reguart N., Ramirez J., Filella X., Molins L. and Agusti A. (2016). "Assessment of a Combined Panel of Six Serum Tumor Markers for Lung Cancer." Am J Respir Crit Care Med 193(4): 427-437.
  - Molina R., Filella X., Trapé J., Augé J. M., Barco A., Cañizares F., Colomer A., Fernandez A., Gaspar M. J., Martinez-Peinado A., Pérez L., Sánchez M., Escudero J. M. (2013). "Principales causas de falsos positivos en los resultados de marcadores tumorales en suero." Sociedad Española de Bioquímica Clínica y Patología Molecular. Comisión de Marcadores Biológicos del Cáncer.



ROC Curve for the MBDAA Test for Lung Cancer



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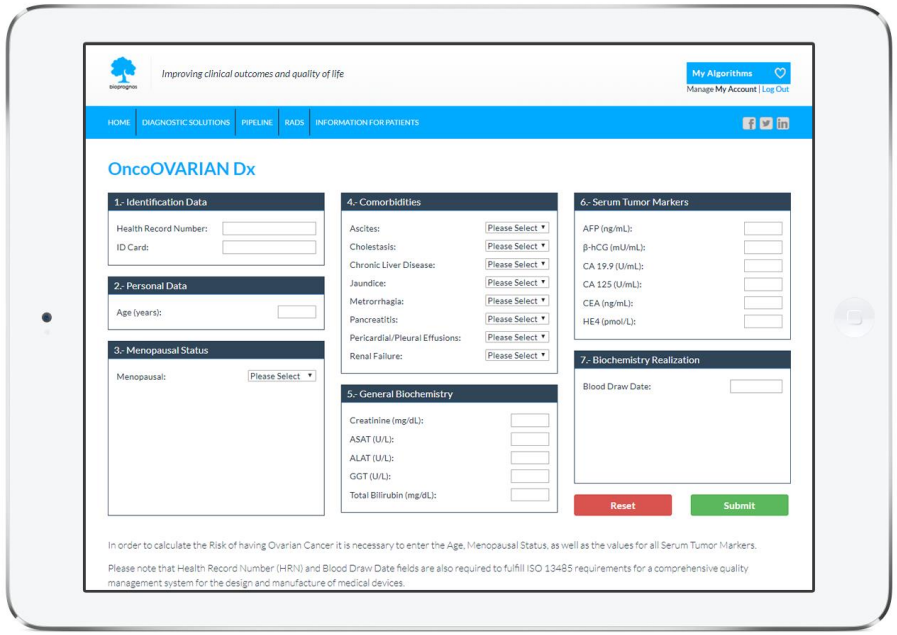
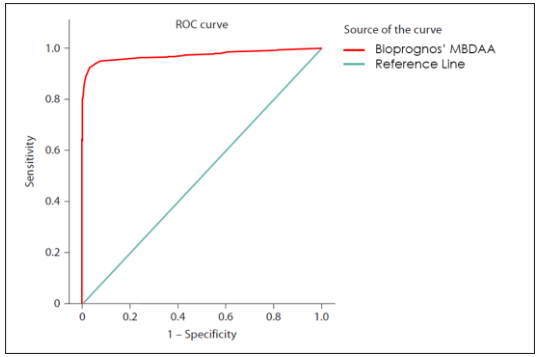
MBDAA Tests to help in Cancer Diagnosis: Ovarian Cancer

- Panel consisting of 6 TM:
  - AFP, β-hCG, CA 19.9, CA 125, CEA and HE4.
- 8 comorbidities associated with false positives in the TM used in this panel:
  - Ascites, Cholestasis, Chronic Liver Disease, Jaundice, Pancreatitis, Pericardial Effusions, Pleural Effusions and Renal Failure.
- Addressed to:
  - Women with adnexal masses suspicious of malignancy.
  - Women with previous image findings suspicious of Ovarian Cancer that should be biopsied to verify malignancy.
- Developed from research:
  - Molina, R., Auge J. M., Escudero J. M., Filella X., Foj, L., Torné A., Lejarcegui J., Pahisa J. "HE4 a novel tumour marker for ovarian cancer: comparison with CA 125 and ROMA algorithm in patients with gynaecological diseases." Tumour Biol, 2011. 32(6): p. 1087-95.
  - Santotoribio J.D., Garcia-de la Torre A., Cañavate-Solano C., Arce-Matute F., Sanchez-del Pino M.J., Perez-Ramos S. "Cancer antigens 19.9 and 125 as tumor markers in patients with mucinous ovarian tumors." EJGO European Journal of Gynaecological Oncology.
  - Shaikh N. A., Memon F., Samo R. P. "Tumor markers; efficacy of CA-125, CEA, AFP and Beta-HCG. An institutional based descriptive & prospective study in diagnosis of ovarian malignancy." Professional Med J 2014;21(4):621-627.
  - Sørensen S. S., Mosgaard B. J. "Combination of CA 125 and CEA can improve ovarian cancer diagnosis." DANISH MEDICAL BULLETIN. Dan Med Bul 2011;58(11):A4331. November 2011.
  - Molina R., Filella X., Trapé J., Augé J. M., Barco A., Cañizares F., Colomer A., Fernandez A., Gaspar M. J., Martinez-Peinado A., Pérez L., Sánchez M., Escudero J. M. (2013). "Principales causas de falsos positivos en los resultados de marcadores tumorales en suero." Sociedad Española de Bioquímica Clínica y Patología Molecular. Comisión de Marcadores Biológicos del Cáncer.



ROC Curve for the MBDAA Test for Ovarian Cancer



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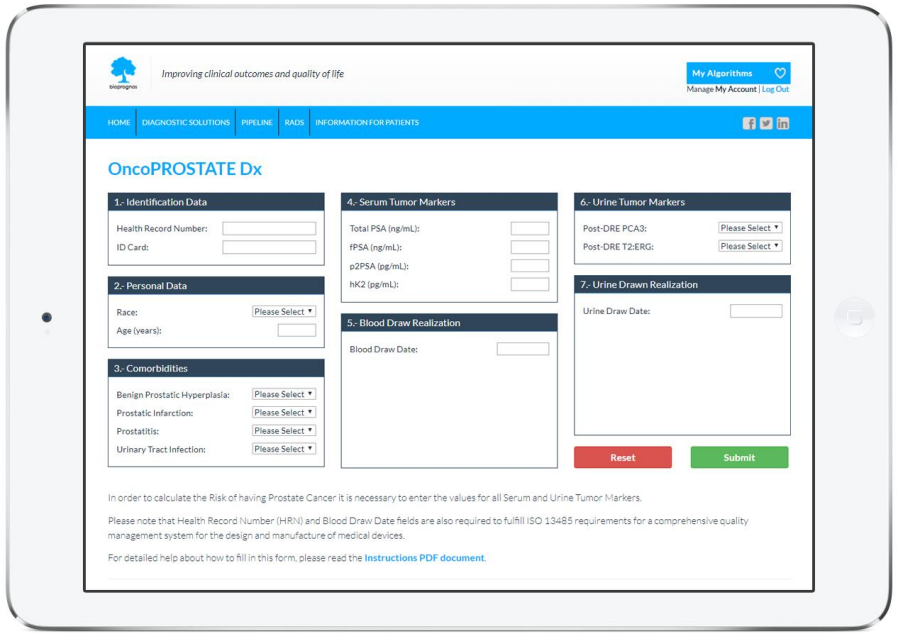
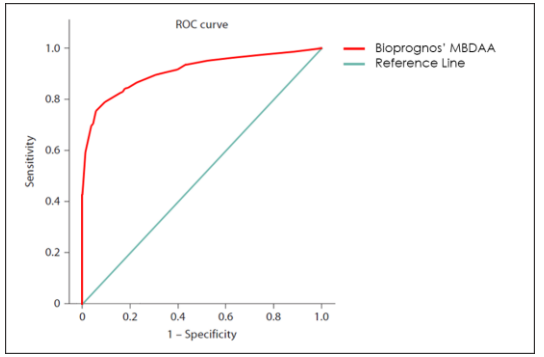
MBDAA Tests to help in Cancer Diagnosis: Prostate Cancer

- Panel consisting of 6 TM:
  - PSA Total, PSA Libre, p2PSA, hK2, PCA3 and T2-ERG.
- 4 comorbidities associated with false positives in the TM used in this panel:
  - Benign Prostatic Hyperplasia, Prostate Infarction, Prostatitis and Urinary Tract Infection.
- Addressed to:
  - Men older than 50 years with PSA blood levels higher than 4 ng/mL.
  - Patients with previous image findings suspicious of Prostate Cancer that should be biopsied to verify malignancy.
- Developed from research:
  - Salami S. S., Schmidt F., Laxman B., Regan M. M., Rickman D. S., Scherr D., Bueti G., Siddiqui J., Tomlins S. A., Wei J. T., Chinnaiyan A, Rubin M. A., Sanda M. G.“ Combining Urinary Detection of TMPRSS2:ERG and PCA3 with Serum PSA to Predict Diagnosis of Prostate Cancer.” Urol Oncol. 2013 July ; 31(5): 566–571.
  - Molina R., Filella X., Trapé J., Augé J. M., Barco A., Cañizares F., Colomer A., Fernandez A., Gaspar M. J., Martinez-Peinado A., Pérez L., Sánchez M., Escudero J. M. (2013). “Principales causas de falsos positivos en los resultados de marcadores tumorales en suero.” Sociedad Española de Bioquímica Clínica y Patología Molecular. Comisión de Marcadores Biológicos del Cáncer.



ROC Curve for the MBDAA Test for Prostate Cancer



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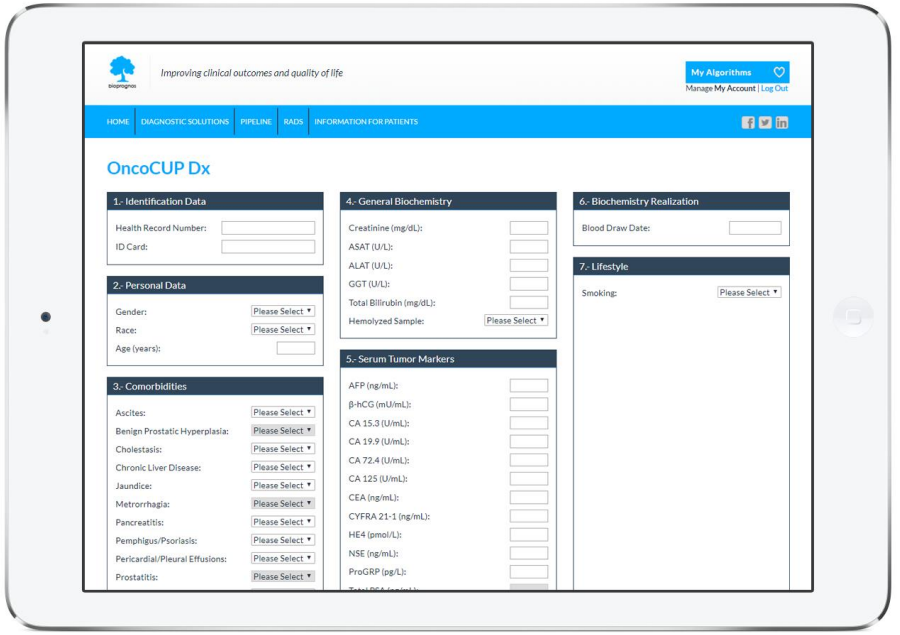
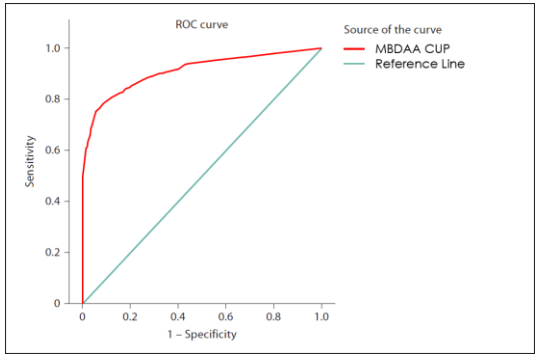
MBDAA Tests to help in Cancer Diagnosis: Cancer Unknown Primary

- Panel consisting of 15 TM:
  - AFP, β-hCG, CA 15.3, CA 19.9, CA 72.4, CA 125, CEA, CYFRA, HE4, NSE, ProGRP, PSA Total, PSA Libre, SCC and S100.
- 12 comorbidities associated with false positives in the TM used in this panel:
  - Ascites, Benign Prostatic Hyperplasia, Cholestasis, Chronic Liver Disease, Jaundice, Pancreatitis, Pemphigus, Pericardial Effusions, Pleural Effusions, Prostatitis and Psoriasis and Renal Failure.
- Lifestyle associated with false positives in the TM used in this panel:
  - Smoking habits.
- Addressed to:
  - Patients with paraneoplastic syndrome and/or suspicion of advanced neoplasia, which the clinician can focus on tests that confirm the diagnosis, avoiding others to look for chronic or other benign diseases.
  - Patients with cancer found in one or more metastatic sites but the primary site can not be determined.
- Developed from research:
  - Molina, R., Bosch X., Auge J. M., Filella X., Escudero J. M., Molina V., Sole M. and Lopez-Soto A. (2012). "Utility of serum tumor markers as an aid in the differential diagnosis of patients with clinical suspicion of cancer and in patients with cancer of unknown primary site." Tumour Biol 33(2): 463-474.
  - Trapé, J. and Molina R. (2006). "Aspectos generales de los marcadores tumorales." JANO 1620: 45-48.
  - Molina R., Filella X., Trapé J., Augé J. M., Barco A., Cañizares F., Colomer A., Fernandez A., Gaspar M. J., Martinez-Peinado A., Pérez L., Sánchez M., Escudero J. M. (2013). "Principales causas de falsos positivos en los resultados de marcadores tumorales en suero." Sociedad Española de Bioquímica Clínica y Patología Molecular. Comisión de Marcadores Biológicos del Cáncer.



ROC Curve for the MBDAA Test for Cancer Unknown Primary



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