A BLOOD GENE EXPRESSION-BASED TEST FOR EARLY DETECTION OF COLORECTAL CANCER : AN INTERNATIONAL MULTI-CENTER CASE-CONTROL STUDY

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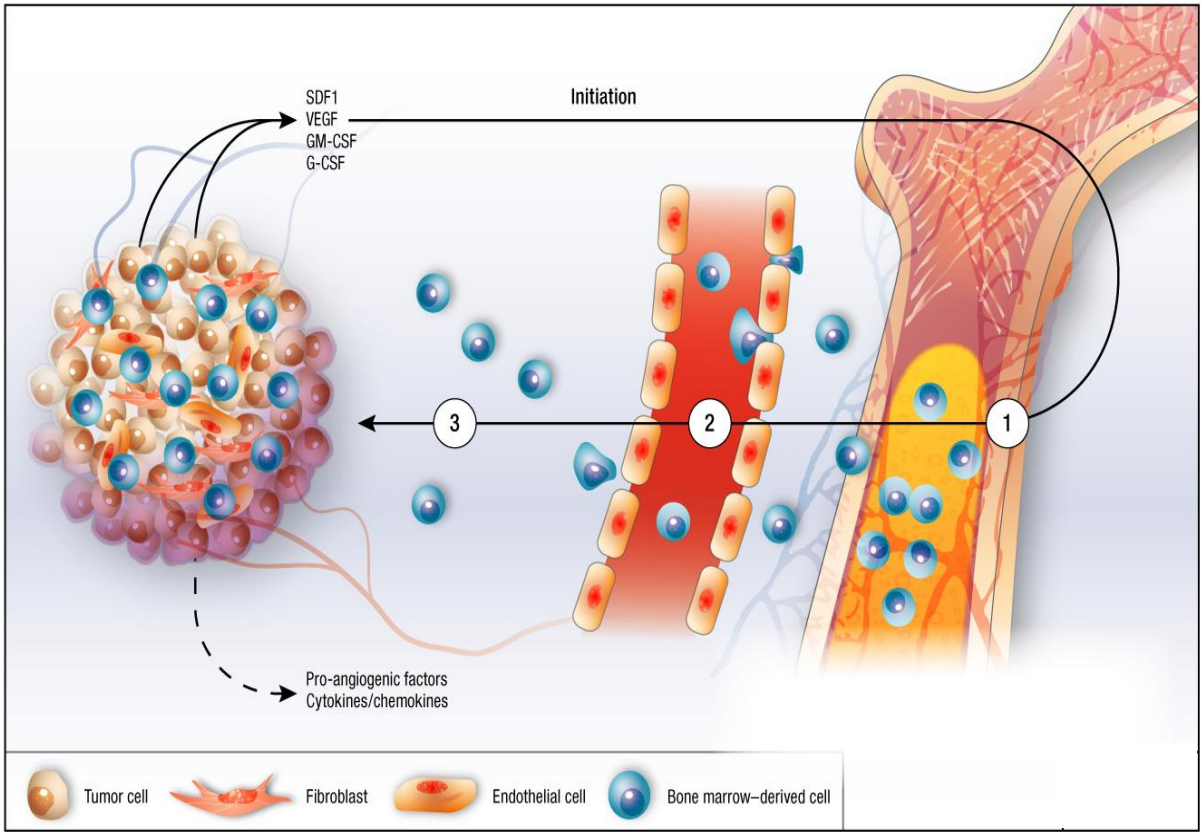
Introduction

Colorectal cancer (CRC):

- ✓ 3rd most common cancer worldwide
- ✓ Often curable, when diagnosed at early stages
- ✓ Non-invasive, highly compliant and robust primary screening tests should be developed
- ✓ A pilot study demonstrated the feasibility of developing a test for CRC and adenomas detection from blood gene expression profiles (*DDW 2010, UEGW 2010*).

Tumor-Host Interaction

Early response to the tumour formation



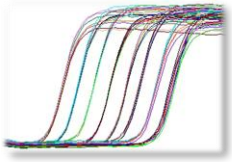
Aim of the Study

To develop a predictive multi-gene multi-classifier algorithm able to differentiate patients with CRC and adenomas from healthy controls.

Test Workflow


✓ **Sample Collection**
- Blood draw in 4 ml Vacutainer® CPT tubes
- PBMC purification within 6 hours


✓ **RNA extraction and cDNA synthesis**

✓ **Real-time PCR**
- Hydrolysis Probe Assays specific for 29 locked biomarkers + 3 housekeeping genes
- Preloaded on 384-well plates.
- Roche LightCycler 480 instrument

DGNP-COL-0310 : Study design

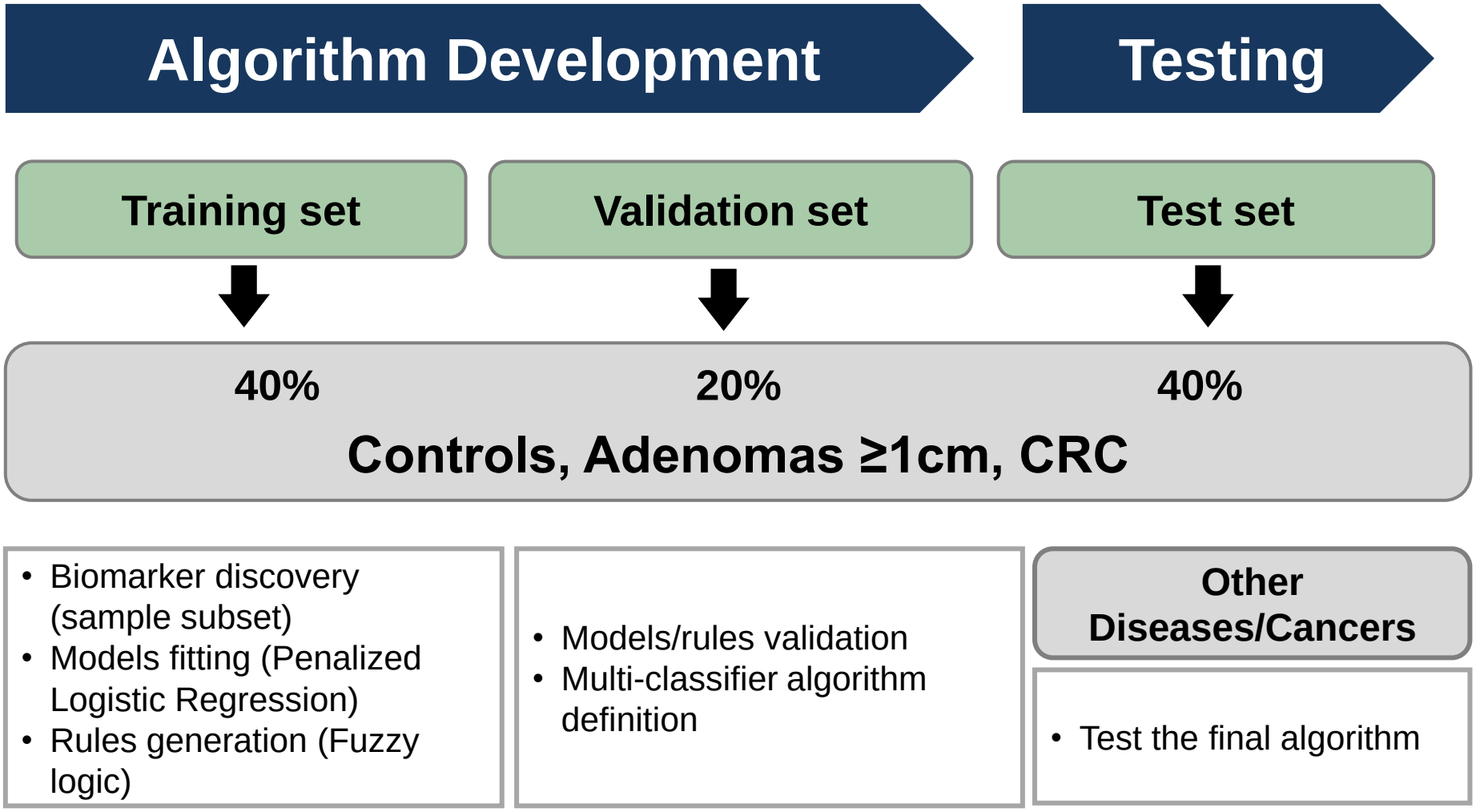
- ✓ **A multi-center case-control study:** 1333 subjects, 2010-2013
  Switzerland (N= 702): 6 centers
  South Korea (N= 619): 3 centers

- ✓ **Main Patient Groups**
  - Controls
  - Adenomas >1cm
  - CRCs
  - Other benign diseases (inflammatory, infectious, gastrointestinal, genito-urinary)
  - Cancer other than CRC
  - Co-morbidities (Subjects who had more than one group-defining disease)

- ✓ **Main exclusion criteria**
  - first-degree family member with CRC <50y or a known predisposition for CRC
  - blood transfusion within the last 30 days

- ✓ **Study procedures**
  - Colonoscopy performed in all subjects (except for other cancers group)
  - Blood draw (4x4ml)
  - Histopathological evaluation by a central pathology board

Statistical design

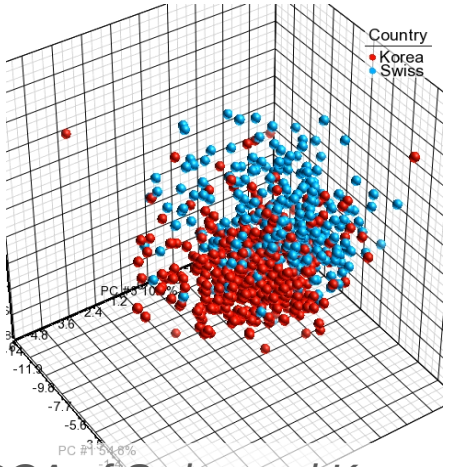


Study population characteristics

	Swiss			Korean		
	Patients	Age (mean)	Male %	Patients	Age (mean)	Male %
Controls	**124**	60.7	45.2	**99**	57.8	44.4
CRC	**74**	69.5	62.1	**129**	62.1	73.7
I	20	70.7	65	40	61.7	82.5
II	15	70.3	60	28	66.8	71.4
III	21	68	57.1	29	60.3	62.1
IV	18	69.3	66.7	32	60.1	75
Unknown	8	70.4	87.5	4	63.3	75
Adenoma ≥1cm	**100**	67.4	64	**154**	60.8	63
Adenoma <1cm	62	65.5	77.4	37	60.5	73
Hyperplastic Polyps	56	60.6	58.9	7	55.7	42.9
Other cancers	63	67.2	71.4	0	/	/
Other diseases	53	63.1	38.9	56	58.5	21.1
Non per-protocol subjects	170	64.8	54.7	137	60.3	53.3

Results

- ✓ **Gender:** No differences in gene expression
- ✓ **Age:** No differences in gene expression
- ✓ **Country:** Significant differences in gene expression between Korean and Swiss samples



PCA of Swiss and Korean datasets

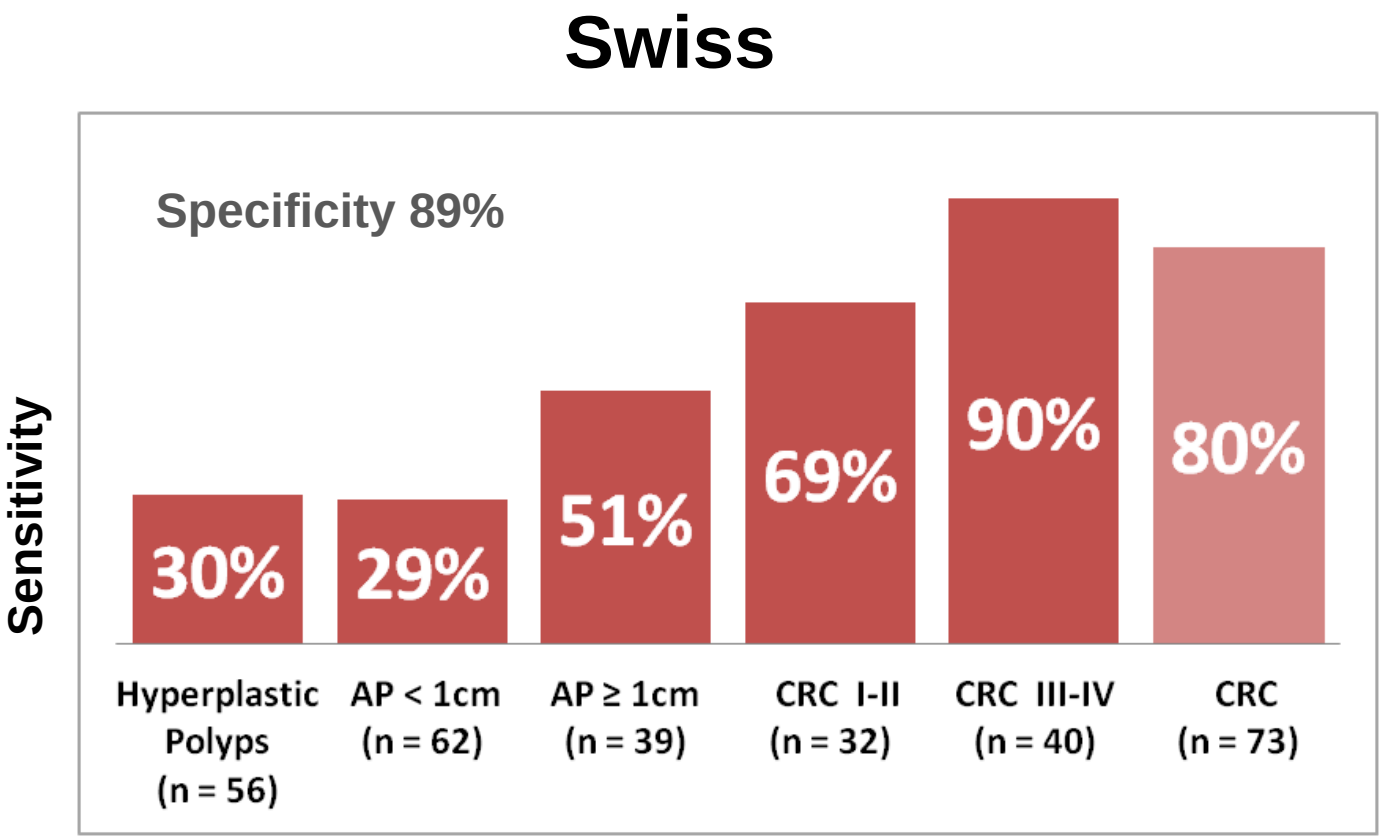
Development of 2 algorithms: Korean and Swiss

COLOX Biomarkers

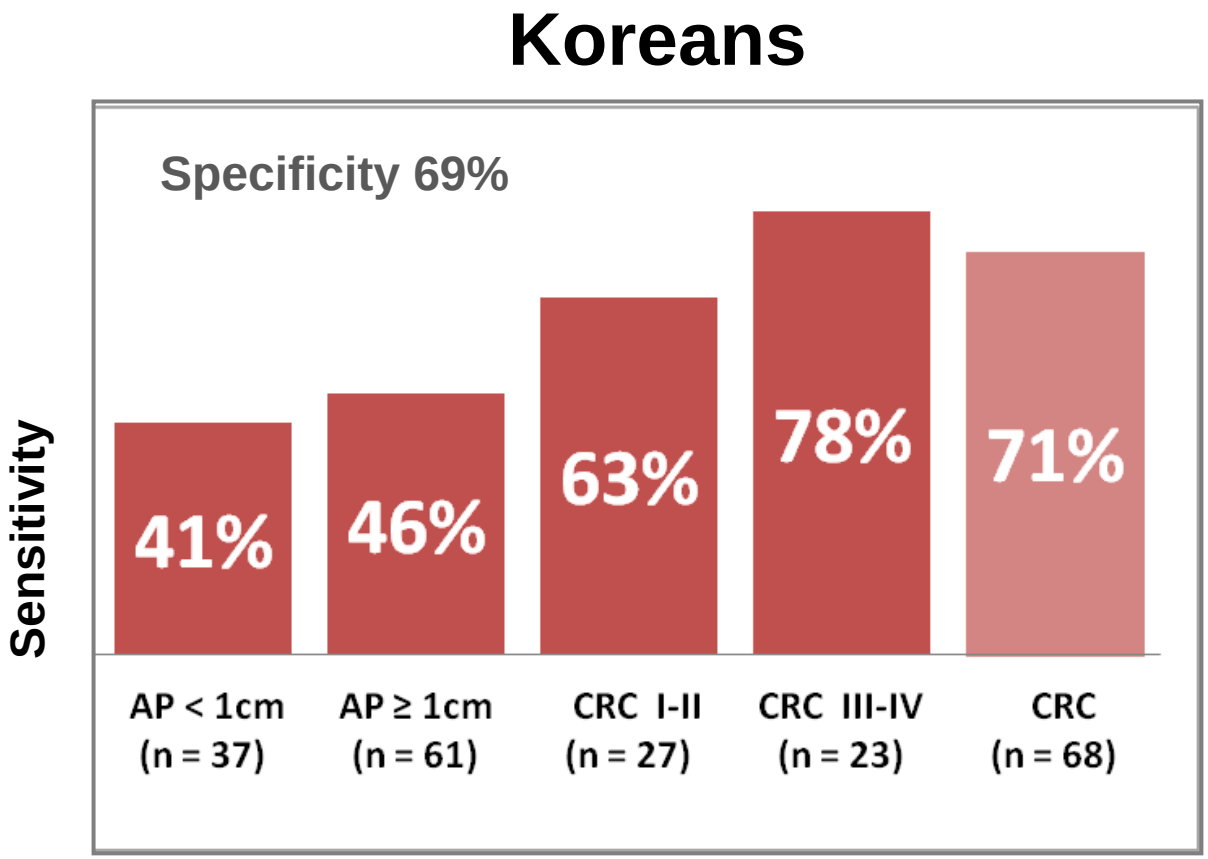
15 most significant biomarkers out of 29 included

Gene Symbol	Biological Function	Swiss		Korean	
		p-value CRC vs CON (Wilcoxon)	Fold Change in CRC	p-value CRC vs CON (Wilcoxon)	Fold Change in CRC
S100A8	Immune Response / Inflammation / Chemotaxis	5.07E-06	1.65	3.68E-02	1.29
IL1B	Immune Response / Inflammation / Chemotaxis	4.19E-04	2.14	6.76E-04	1.75
CCR1	Cell adhesion / Chemotaxis	4.42E-04	1.65	6.25E-02	1.23
PTGS2	Inflammation	7.68E-04	2.11	1.99E-03	1.87
PPARG	Transcription / Cell cycle / Regulation	3.59E-03	1.41	3.44E-03	1.31
MAPK6	Transcription / Cell cycle / Regulation	3.95E-03	1.15	3.00E-02	1.07
TNFSF13B	Immune Response / Inflammation / Chemotaxis	1.03E-02	1.21	5.53E-02	1.14
CACNB4	Ion transport	1.31E-02	-1.30	2.07E-01	-1.10
MMP11	Collagen degradation	1.66E-02	-1.30	6.25E-02	-1.17
LTF	Ion transport	2.14E-02	2.36	6.47E-02	1.75
CD63	Differentiation / Structure	3.14E-02	1.14	1.06E-02	1.14
CES1	Immune Response / Inflammation / Chemotaxis	5.70E-02	1.18	4.29E-01	-1.23
CXCL10	Immune Response / Inflammation / Chemotaxis	7.13E-02	-1.29	8.28E-01	-1.06
MAP2K3	Differentiation / Structure	9.89E-02	1.09	1.19E-02	1.24
MMP9	Collagen degradation	1.21E-01	1.35	3.79E-03	1.67

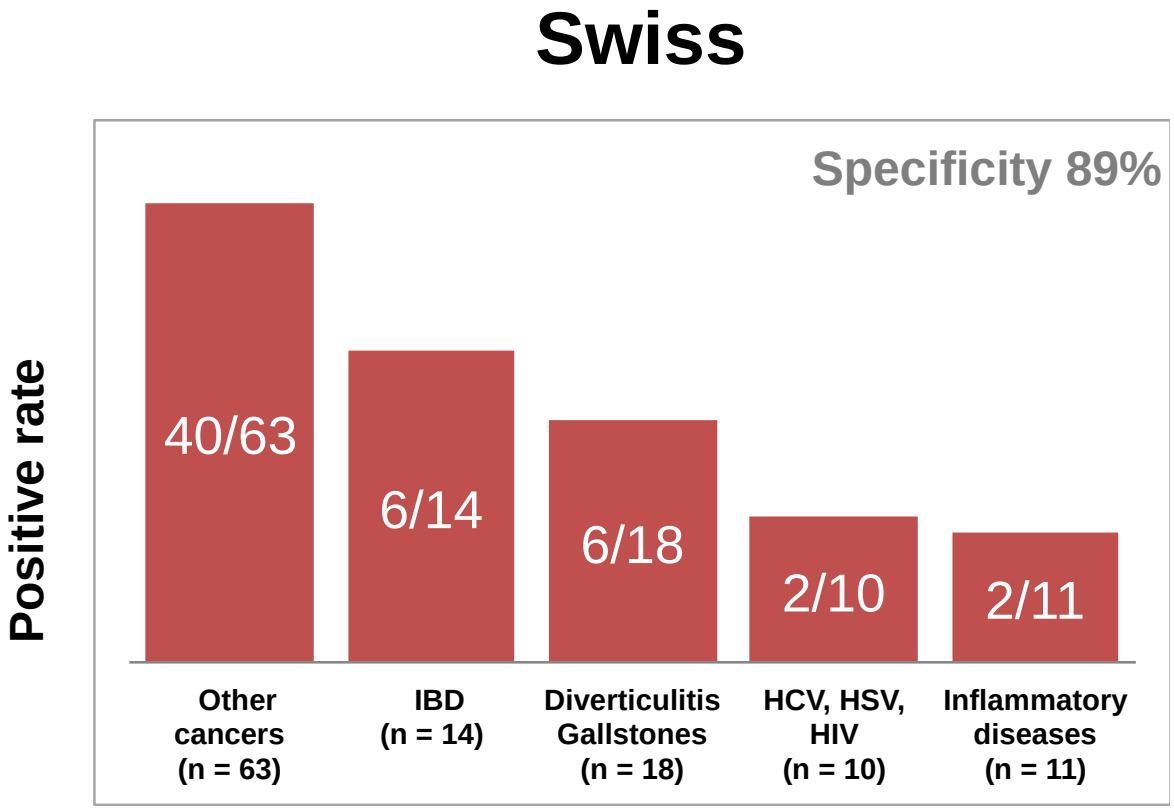
Results: Test Set



Results: Test Set



Results: benign diseases and other cancers



Conclusions

- ✓ We developed an accurate, **blood-based test** (COLOX®) for detecting the presence of CRC and pre-neoplastic lesions.
- ✓ At a **specificity of 89%,** COLOX® showed a **sensitivity of 80%** and **51%** for CRC and AP ≥ 1cm detection, respectively, for the Swiss population.
- ✓ A **Swiss and a Korean algorithm** were developed separately because of the observed differences in gene expression. The disparities might depend on genetic and/or environmental factors and will be investigated in a separate study.
- ✓ A large **comparison study** between COLOX® and a commercially available FIT is in preparation

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