

Supplementary data 1: Primer sequences used in qPCR experiments

Name	Sequence	Annealing temperature
FKBP52 forward	5'-TGGTAGGGATGAGGTCTGATAAGA-3'	60°C
FKBP52 reverse	5'-AGGGAAAGGATGAGGAAAACG-3'	60°C
PPIA forward	5'-CCTGGTGGTGCATGCCTAGT-3'	60°C
PPIA reverse	5'-CTCACTCTAGGCTCAAGCAATCC-3'	60°C
PRDX2 forward	5'-TGTGATCGTCCGTGCGTCTA-3'	60°C
PRDX2 reverse	5'-CGATGCGCGCGTTAC-3'	60°C
TBP89 forward	5'-TTTTCTTGCTGCCAGTCTGGAC-3'	65°C
TBP89 reverse	5'-CACGAACCACGGCACTGATT-3'	65°C
RS9 forward	5'-AAGGCCGCCCGGGAAGTCTGAC-3'	62°C
RS9 reverse	5'-ACCACCTGCTTGCGGACCCTGATA-3'	62°C

Supplementary data 2: Sensitivity, specificity, predictive values and diagnosis accuracy of the 5 markers in cancer, PBC and CIS.

Marker	FKBP52			PPIA			PRDX2			HSP60			MUC1		
Comparison	HC/cancer	HC/PBC	HC/CIS	HC/cancer	HC/PBC	HC/CIS	HC/cancer	HC/PBC	HC/CIS	HC/cancer	HC/PBC	HC/CIS	HC/cancer	HC/PBC	HC/CIS
Se	50.1	43.1	54.8	49.6	48.2	51.3	45.2	45.4	45.1	35.0	43.7	27.3	36.5	42.5	31.9
Sp	87.2	89.5	86.7	87.3	88.8	85.7	85.5	87.4	84.0	87.1	86.1	89.5	87.9	86.9	88.6
PPV	85.7	72.6	78.4	85.6	73.5	76	82.6	69.9	71.3	80.5	67.0	69.7	82.1	67.7	71.2
NPV	53.4	70.9	68.5	53.2	72.7	66.6	50.6	71.3	63.4	46.8	70.3	58.2	47.6	70.1	59.6
Diagnostic accuracy	64.8	71.3	71.8	64.5	72.9	69.6	61.1	70.9	65.8	55.6	69.5	60.4	56.8	69.5	62.0

Values are represented in %.

Se (sensitivity) = number of true positive / number of cases

Sp (specificity) = number of true negative / number of controls

PPV (positive predictive value) = $\frac{\text{prv} \times \text{Se}}{\text{prv} \times \text{Se} + (1 - \text{prv}) \times (1 - \text{Sp})}$ where “prv” corresponds to the Prevalence of the disease, estimated by the proportion of diseased subjects in the population.

NPV (negative predictive value) = $1 - \frac{\text{prv} \times (1 - \text{Se})}{\text{prv} \times (1 - \text{Se}) + (1 - \text{prv}) \times \text{Sp}}$

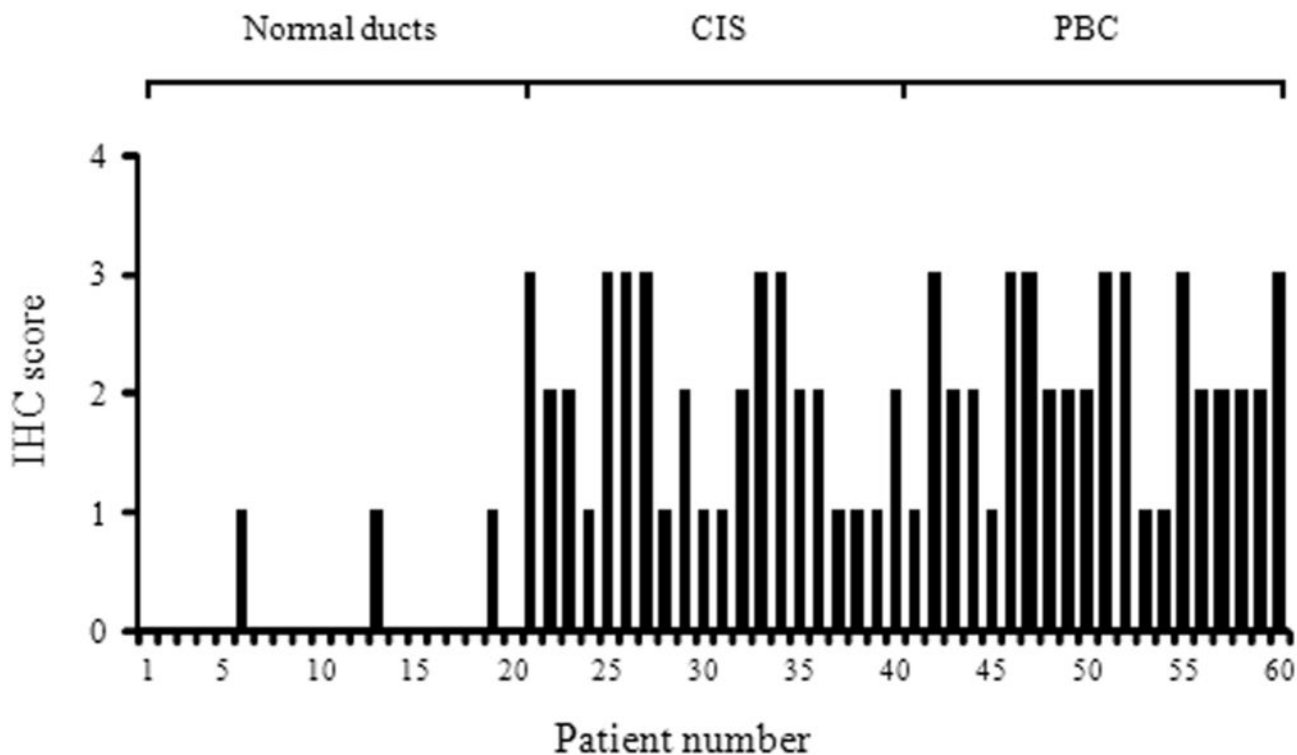
Diagnostic accuracy = (number of true positive + number of true negative) / total number of samples.

Supplementary data 3: Leave-one-out cross-validation*

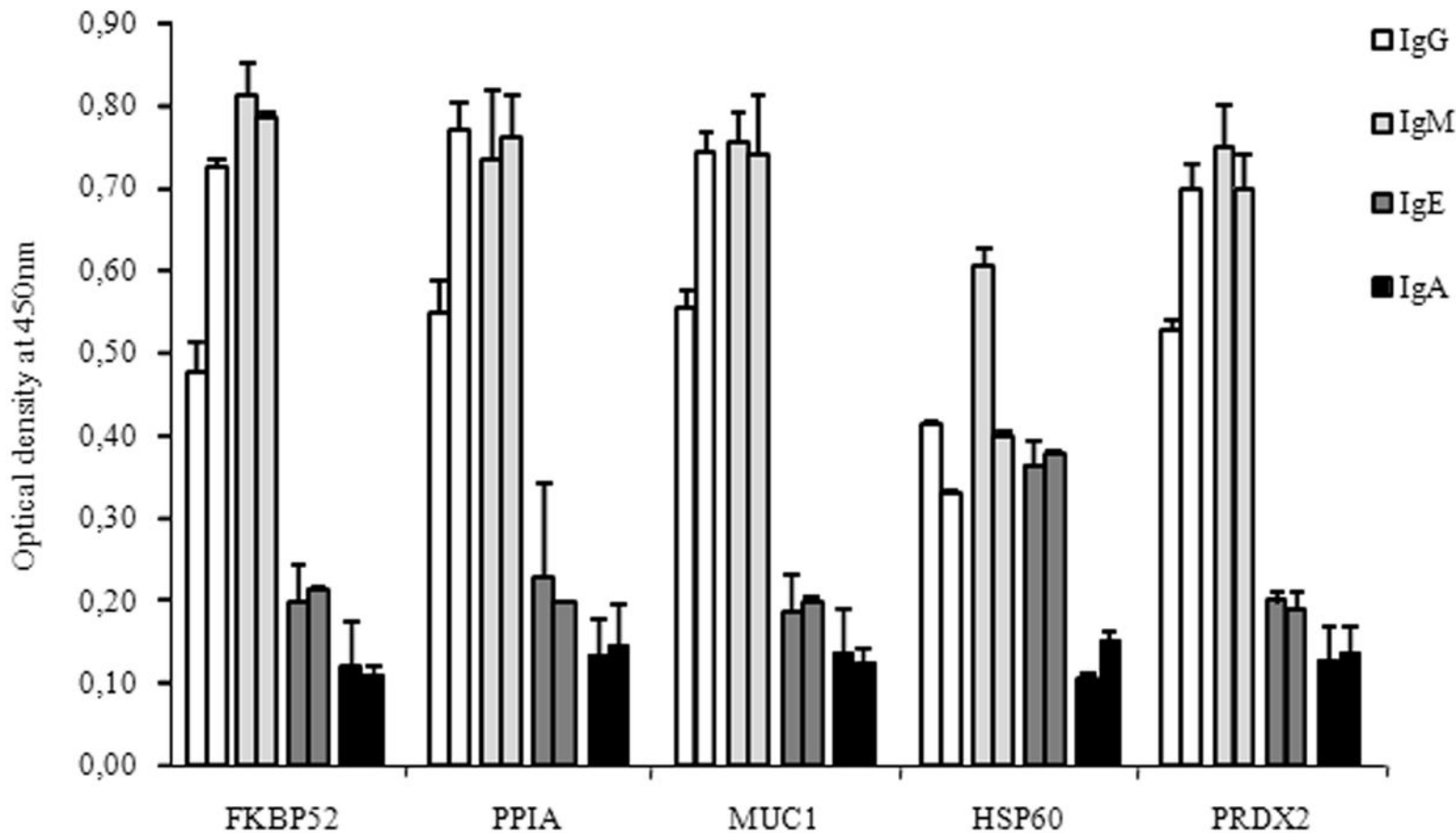
Comparison	Sensitivity (%)	Specificity (%)	Diagnostic accuracy** (%)
Cancer vs Healthy	52.1	77.4	62.1
PBC vs Healthy	53.3	87.1	73.8
CIS vs Healthy	70.7	76.3	73.7

*One sample was removed from the statistical model containing a total of 235 samples (respectively 153 and 175) and a classifier was generated to predict the status, healthy or cancer (respectively PBC and CIS), of the removed sample using the rest of the samples. This procedure was repeated for all samples.

**Diagnostic accuracy = (number of true positive + number of true negative)/total number of samples.



Supplementary data 4: Variations in FKBP52 protein expression in normal ducts, CIS and PBC by IHC. IHC scores were determined as described in ‘patients and methods’ section.



Supplementary data 5: Characterisation of human Ig subclasses of autoantibodies. Two pools of 5 sera positive for each autoantibody were subjected to ELISA assays to determine Ig subclass of autoantibodies, according to the procedure described in material and methods. As secondary antibodies, we used an anti-human IgG, γ chain specific (#A6029, Sigma), an anti-human IgM, $Fc_{5\mu}$ fragment specific (#109-035-043, Jackson Immunoresearch Laboratories), an anti-human IgE, ϵ chain specific (#A9667, Sigma), or an anti-human IgA, α chain specific (#A0295, Sigma). Each pool was assayed in duplicate in 2 separate experiments. Results are presented as the mean of $OD \pm SD$ for each pool.