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**Basic concept of latent class analysis**

Consider the following observed outcomes of three tests:

|  |  |  |  |
| --- | --- | --- | --- |
|  | Test 1 | Test2 | Test 3 |
| Subject 1 | + |  | + |
| Subject 2 |  | + | + |
| .. | … | … | … |

The probability of this outcome depends on the prevalence of the disease and on the characteristics of each test. Let denote the true antibody status which is not directly observable with D = 1 and D = 0 describing an antibody-positive status and an antibody-negative status, respectively. The prevalence of the antibody is. P(Tk=+|D=0) is the probability of a positive result of test k, when the true antibody status is negative, i.e. the false positive rate. Similarly the following notation is applied:

* P(Tk=|D=0) = 1  P(Tk=+|D=0) is the true negative rate for test k
* P(Tk=|D=1) = 1  P(Tk=+|D=1) is false negative rate for test k
* P(Tk=+|D=1) = 1  P(Tk=|D=1) is the true positive rate for test k
* P(Tk=+|D=0) = 1  P(Tk=|D=0) is the false positive rate for test k

The joint probability of the observed test outcome for the first two subjects can be expressed as

Pobserved  =

*p* \* P(T1=+|D=1) \* P(T2=|D=1)\* P(T3=+|D=1) + // first subj., true status pos.

(1*p*) \* P(T1=+|D=0) \* P(T2=|D=0) \* P(T3=+|D=0) + // first subj., true status neg.

*p* \* P(T1=|D=1) \* P(T2=+|D=1) \* P(T3=+|D=1) + // sec. subj., true status pos.

(1*p*) \* P(T1=|D=0) \* P(T2=+|D=0) \* P(T3=+|D=0) // sec. subj., true status neg.

=

*p* \* P(T1=+|D=1) \* (1P(T2=+|D=1)) \* P(T3=+|D=1) +

(1*p*) \* P(T1=+|D=0) \* (1P(T2=+|D=0)) \* P(T3=+|D=0) +

*p* \* (1P(T1=+|D=1)) \* P(T2=+|D=1) \* P(T3=+|D=1) +

(1*p*) \* (1P(T1=+|D=0)) \* P(T2=+|D=0) \* P(T3=+|D=0)

For latent class analysis a joint probability function to calculate Pobserved can be stated that incorporates all observed outcomes from all investigated subjects. The prevalence of the latent class and two parameters of each test (P(Tk=+|D=1), P(Tk=+|D=0)) are the required parameters for this function.

Latent class analysis assumes that the observed outcome is also the most likely outcome. Several methods like the expectation–maximization (EM) algorithm exists to find the parameter set with the highest probability for Pobserved. This parameter set constitutes the result of the latent class analysis.

The most likely true antibody status of each subject is then estimated using this parameter set. If for subject 1 the probability of an antibody-positive status is higher than the probability of an antibody-negative status , subject 1 is regarded as positive for subsequent analysis, i.e. antibody-positive would constitute the “gold-standard” status of this subject. The same principle is applied to all other subjects of the study. Sensitivity of each test k equates directly to the true positive rate P(Tk=+|D=1). Specificity is the true negative rate P(Tk= |D=0) = 1  P(Tk=+|D=0).

**SPR antiphospholipid assays**

**Chemicals**

SIA Kit Au containing gold-coated glass slides, BIAcore amine coupling kit and ethanolamine-HCl were bought from GE Healthcare (Freiburg, Germany). 11-mercaptoundecanoic acid, 11-mercaptoundecan-1-ol, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) and human transferrin were purchased from Sigma-Aldrich (Taufkirchen, Germany). The amine-functionalized cardiolipin derivative (-)-1-*O*-(1’-*O*-[12’’-aminododecanoyl]-2’-*O*-hexadecanoyl-*sn*-glycer-3’-yl-*O*-phosphoryl)-3-*O*-(1’,2’-di-*O*-hexadecanoyl-*sn*-glycer-3’-yl-*O*-phosphoryl)-*sn*-glycerol (amino-CL) was synthesized as described in (1). Human β2-GPI was purchased from Scipac (Sittingbourne, UK). According to the manufacturer’s information, the applied β2-GPI was purified from human serum by the following consecutive steps: precipitation by addition of ethanol - precipitation by addition of perchloric acid - ion exchange chromatography - affinity chromatography (protein A) - gel filtration chromatography. Fetal calf serum (FCS) was from PAA Laboratories GmbH (Pasching, Austria), KCl from Roth (Karlsruhe, Germany) and all other chemicals from Merck (Darmstadt, Germany).

**Preparation of SPR surfaces and serum measurements**

A BIAcore X device (GE Healthcare) at a working temperature of 25 °C was used for SPR experiments. Preparation of the aβ2-GPI- and the aCL-surface and the respective serum measurements were basically performed as described previously (2, 3). For purposes of unification, however, both protocols were slightly modified.

Briefly, gold surfaces of the SIA Kit Au sensor chips were coated with a self-assembled monolayer by an over-night incubation in a 10 mmol/L solution of 11-mercaptoundecanoic acid and 11-mercaptoundecan-1-ol in a 6:4 molar ratio. Antigen immobilization for the β2-GPI chip was accomplished by use of the BIAcore amine coupling kit with HBS (20 mmol/L HEPES, 150 mmol/L NaCl, pH 7.3) as running buffer. Surfaces of both the specific flow cell (FC2) and the reference flow cell (FC1) were activated with 35 µL of a 100 mmol/L 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and 25 mmol/L N-hydroxysuccinimide solution at 5 µL/min. For immobilization of β2-GPI on FC2, flow was reduced to 2 µL/min and 75 µg of β2-GPI in 100 µl HBS were injected. FC1 was prepared by injecting 60 µL of 12.5 µg/µL human transferrin in HBS at 3 µL/min as non-APS-antigenic protein. The aCL chip was prepared similarly with an injection of 100 µl of a 74 nmol/L solution of amino-CL diammonium salt instead of β2-GPI. The amino-CL immobilization was – additionally to the protocol described above - followed by a subsequent injection of 40 µL 1 mol/L ethanolamine-HCl pH 8.5 as well at 2 µL/min.

Serum measurements were performed at a flow rate of 10 µL/min with PBS (2.6 mmol/L KCl, 138 mmol/L NaCl, 10 mmol/L HNa2PO4∙2H2O, 1.8 mmol/L H2KPO4, pH 7.4) containing 5 % FCS as running and dilution buffer. Fetal calf serum thereby serves as β2-GPI source for the aCL chip to create the generally accepted main target antigen of aCL, i.e. β2-GPI complexed with the anionic cardiolipin. For measurement of aβ2-GPI, 45 µL of 1:100-diluted serum were injected, followed by 300 s of buffer injection for dissociation. The β2-GPI surface was then regenerated by injecting 10 µL 10 mmol/L glycine pH 2.6 twice. aCL were evaluated in 1:10 dilutions of sera, of which as well 45 µl were injected over the surface with a dissociation time of 300 s. In case of the aCL chip, regeneration was achieved with 10 µL of 50 mmol/L NaOH/1 mmol/L NaCl and a subsequent injection of   
10 µL PBS. To correct for refractive bulk effects, unspecific binding phenomena and baseline drift, net sensorgrams were calculated as difference of the signals of the specific and the reference flow cell, expressed in arbitrary resonance units (RU). Maximum binding of the sera was evaluated as the signal difference FC2 - FC1 at the end of the 270 s long association phase.

**References**

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