

High Titer of Anticardiolipin Antibody Is Associated with First-Ever Ischemic Stroke in Taiwan

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High Titer of Anticardiolipin Antibody Is Associated with First-Ever Ischemic Stroke in Taiwan

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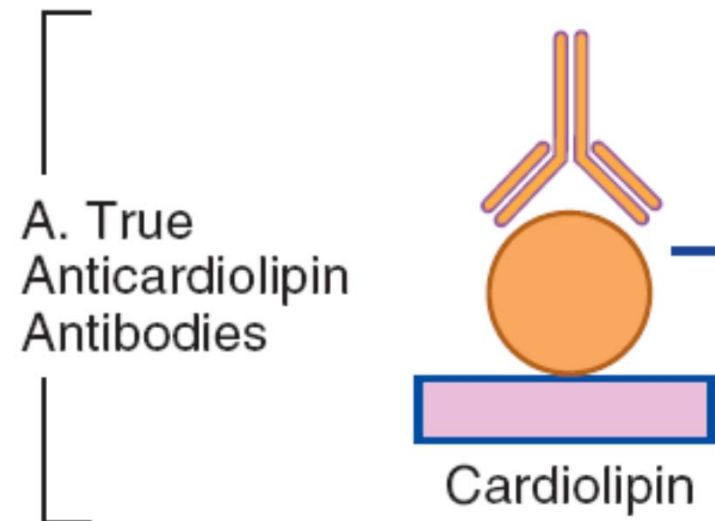
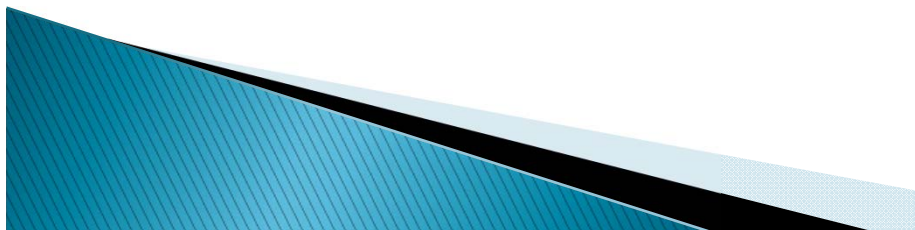
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Introduction



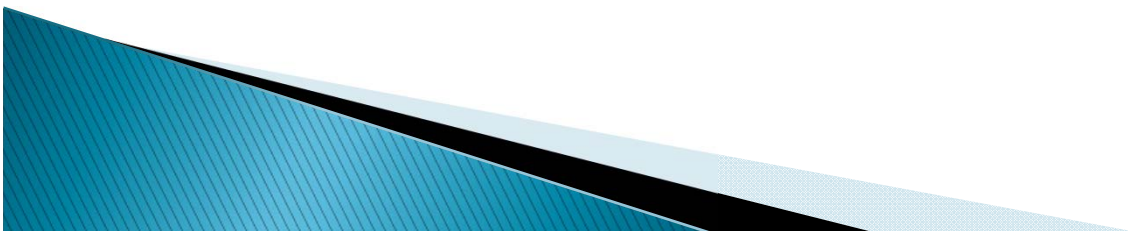
Anticardiolipin antibodies (aCL)

- ▶ Antiphospholipid antibodies
- ▶ Autoantibodies
- ▶ Thrombo-occlusive events (IgG isotype)
 - Cerebrovascular system (most)
- ▶ aCL↑, stroke patients under 40 years old
- ▶ aCL↑, future stroke↑



Novelty

- ▶ Not yet established as an independent risk factor for ischemic strokes
- ▶ Case-control study was set up to determine the association between aCL and ischemic stroke among Taiwanese

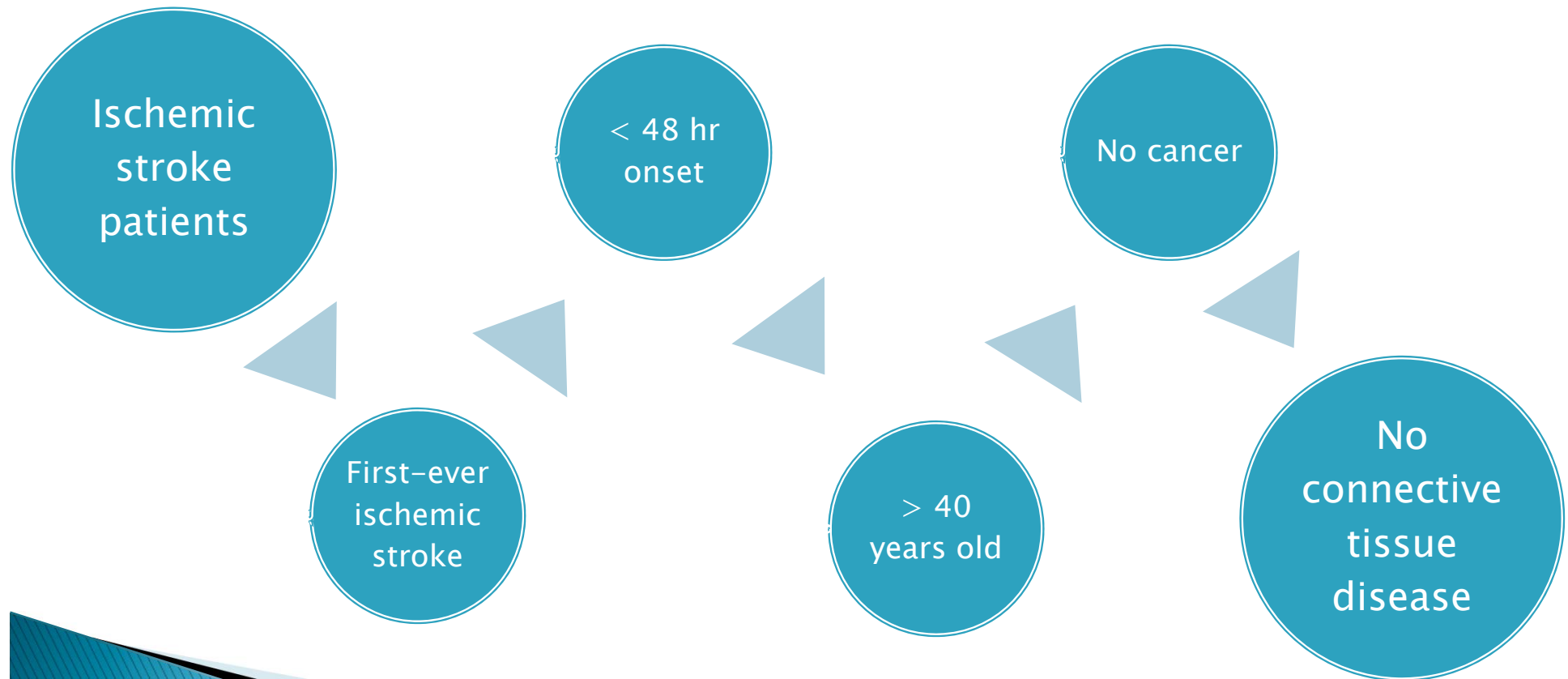


Subjects and Methods



Inclusion Criteria

1996–1999, Shin Kong WHS Memorial Hospital, Neurological wards



- ▶ Response rate = 96.5% (n=273)
Pregnancy, systemic lupus erythematosus,
transient ischemic attack, venous thrombosis
and peripheral vessel thrombosis



- ▶ Control: outpatients (n=181)
 - Other than stroke (nonspecific symptoms)
 - Headache
 - Musculoskeletal pain
 - Peripheral vestibulopathy
 - Parkinson's disease
 - No cancer or connective tissue disease



Determination of aCL



Serum specimens centrifuged and frozen

ELISA

IgG aCL (GPL) and IgM aCL (MPL) levels:

- Negative(<10GPL <7.5MPL), low positive(10–20GPL 7.5–15MPL), high positive(>20GPL >15MPL)

Data analysis

- ▶ Demographics and vascular risk factor
 - χ^2 test: categorical variables
 - Two sample t test: continuous variables
- ▶ Odds ratios:
 - Stroke risk: high positive aCL vs normal/low positive aCL
- ▶ Adjustment (logistic regression):
 - Model I : not adjusted
 - Model II : age, sex
 - Model III : age, sex, smoking, history of diseases



Results



Basic Sample Characteristics

► Table 1. Characteristics of the study participants

	Ischemic stroke patients (n = 273)	Controls (n = 181)	p value
T test			
Mean age	64.9 (12.6)	63.3 (11.0)	0.1506
Gender male	173/273 (62.6)	85/181 (47.2)	0.0012
Cigarette smoking	100/264 (37.9)	99/178 (62.1)	0.0002
Alcohol drinking	50/264 (18.9)	40/178 (22.5)	0.3657
X ² Hypertension	163/263 (62.0)	63/177 (35.6)	<0.0001
Diabetes mellitus	82/263 (31.2)	19/177 (10.7)	<0.0001
Left ventricular hypertrophy	84/263 (31.9)	22/177 (12.4)	<0.0001
Atrial fibrillation	21/263 (8.0)	3/177 (1.7)	0.0044
Hyperlipidemia	46/273 (16.9)	45/181 (24.9)	0.0368
Mean (SD) was presented in age. n (%) was presented in other indices.			

Basic Sample Characteristics

- ▶ There are significant differences in the proportion of gender, cigarette smoking, hypertension, DM, LVH, A-fib, and hyperlipidemia between the control group and ischemic stroke patient

	Ischemic stroke patients (n = 273)	Controls (n = 181)	p value
Mean age	64.9 (12.6)	63.3 (11.0)	0.1506
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Diabetes mellitus	82/263 (31.2) *	19/177 (10.7)	<0.0001
Left ventricular hypertrophy	84/263 (31.9) *	22/177 (12.4)	<0.0001
Atrial fibrillation	21/263 (8.0) *	3/177 (1.7)	0.0044
Hyperlipidemia	46/273 (16.9)	45/181 (24.9) *	0.0368

Mean (SD) was presented in age. n (%) was presented in other indices.

Level of aCL IgG and IgM

- ▶ No significant difference between mean aCL-IgG aCL-IgM is observed applying two sample t test

	Ischemic stroke patients (n = 273)	Controls (n = 181)	p value
Mean aCL-IgG	6.8 (7.7)	5.8 (4.7)	0.1206
Mean aCL-IgM	7.0(8.3)	7.5 (7.6)	0.5289

Value indicates: mean (SD)

Stratified Level of aCL IgG and IgM

- ▶ After stratifying mean aCL-Igs into three groups, no significant difference is observed applying the Chi square test

Table 2. Distribution of aCL

	Ischemic stroke patients (n = 273)	Controls (n = 181)	p value
Levels of aCL-IgG			
Negative (<10)	238 (87.2)	162 (89.5)	0.1351
Low positive (10–20)	23 (8.4)	17 (9.4)	
High positive (>20)	12 (4.2)	2 (1.2)	
Levels of aCL-IgM			
Negative (<7.5)	196 (71.8)	123 (68.0)	0.6609
Low positive (7.5–15)	57 (20.9)	44 (24.3)	
High positive (>15)	20 (7.3)	14 (7.8)	

Calculation of Odds Ratio

- ▶ Determine whether or not subject with high level of aCL-Igs is more likely to develop ischemic stroke
- ▶ Blood aCL-IgG > 20 GPL and aCL-IgM > 15 MPL is the cut off point

	Ischemic Stroke	Control
IgG > 20	12	2
IgG ≤ 20	261	179

$$OR = \frac{12 \times 179}{2 \times 261} = 4.11$$

Calculation of Odds Ratio

- ▶ Calculation of 95% CI, taking aCL-IgG as an example
- ▶ CI of OR is between 0.091 to 18.595, which includes 1
- ▶ Unable to justified that high blood aCL-IgG is a risk factor for ischemic stroke

Calculation of Odds Ratio

- ▶ Method for calculation for 95% CI is the same for aCL-IgM
- ▶ 95% CI of aCL-IgM also contains 1, therefore high aCL-IgM present in blood is not a significant risk factor of ischemic stroke

	OR (95%CI)	p value
ACL IgG ≥ 20 vs. <20	4.11 (0.91–18.60)	0.0662
ACL IgM ≥ 15 vs. <15	0.94 (0.46–1.92)	0.8707

Construction of Different Models

- ▶ As previously mentioned, there is significant different the ratio of gender, smoking status...between the two group
- ▶ Even age is not significantly different between two group, it's widely accepted that advanced age is a risk factor for ischemic stroke
- ▶ In order to avoid interference, two model is constructed in order to eliminate these factors



Different Models

- ▶ Model 1: Unadjusted
- ▶ Model 2: Adjusted by age and sex
- ▶ Model 3: adjusted by age, sex, smoking, and history of hypertension, DM, A-fib, LVH, and hyperlipidemia
- ▶ Method for adjustment: Adjusted Logistic Regression



Adjusted Logistic Regression

- ▶ Unadjusted OR can be expressed as:

$$\ln(\text{OR}) = \beta_0 + \beta_{\text{aCL}}$$

- ▶ In the formula above, β_{aCL} represent the effect of high blood aCL on the risk of developing ischemic stroke
- ▶ If the CI of β_{aCL} contains 0 , calculated OR ($e^{\beta_0 + \beta_{\text{aCL}}}$) is statistically insignificant compare to the OR (e^{β_0}) that did not consider aCL as a risk factor

Adjusted logistic regression

- ▶ In the form of null hypothesis:

$$H_0: \beta_0 = \beta_0 + \beta_{aCL} \quad \beta_{aCL} = 0$$

$$H_1: \beta_0 \neq \beta_0 + \beta_{aCL} \quad \beta_{aCL} \neq 0$$

- ▶ When the CI of β_{aCL} contains 0 , H_0 can not be rejected



Adjusted logistic regression

- ▶ In the case of considering smoking status as a risk factor

- ▶ Unadjusted formula of OR:

$$\ln(\text{OR}) = \beta_0 + \beta_{\text{aCL}}$$

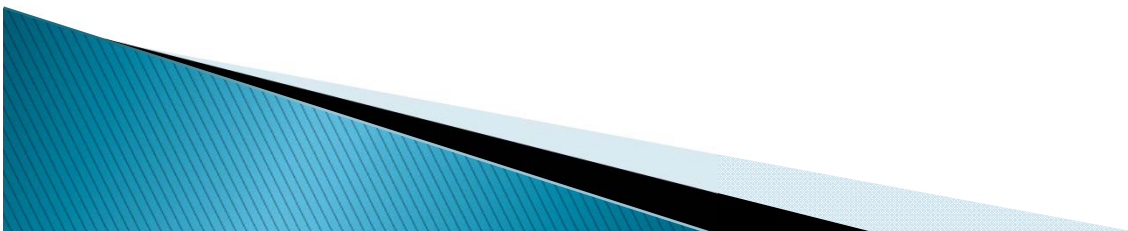
- ▶ After considering smoking status, formula is transformed into :

$$\ln(\text{OR}) = \beta'_0 + \beta'_{\text{aCL}} + \beta'_{\text{smoke}}$$

- ▶ The β'_{aCL} in the second formula is the effect of high blood aCL on the risk of developing ischemic stroke under identical smoking status

Adjusted logistic regression

- ▶ β'_{aCL} and β'_{smoke} have their own CI
- ▶ If the CI of β'_{aCL} contain 0 , no significant effect of high blood aCL is associate with the risk of developing ischemic stroke under identical smoking status
- ▶ The same is true for β'_{smoke}



Adjusted logistic regression

- ▶ OR formula for Model 1:

$$\ln(\text{OR}) = \beta_0 + \beta_{\text{aCL}}$$

- ▶ OR formula for Model 2:

$$\ln(\text{OR}) = \beta'_0 + \beta'_{\text{aCL}} + \beta'_{\text{age}} + \beta'_{\text{sex}}$$

- ▶ OR formula for Model 3:

$$\ln(\text{OR}) = \beta''_0 + \beta''_{\text{aCL}} + \beta''_{\text{age}} + \beta''_{\text{sex}} + \dots + \beta''_{\text{hyperlipidemi}}$$

a

Note: aCL-IgM and aCL-IgG is calculated separately

Adjusted logistic regression

- ▶ From Model 2, the 95% CI of β'_{aCL} contains 0 (CI of β'_{age} and β'_{sex} is unknown)
- ▶ 95% CI of β''_{aCL} derived from Model 3 (only for gG) doesn't contain 0, which imply under identical status (age, sex, smoking status), subject with high aCL-IgG present in blood have a higher odds ratio for developing ischemic stroke

Discussions



Discussions

- ▶ The definition above is based on the reviews of past research (as the following table), and the further Discussions will also compare with this table.

Author	Ischemic stroke patients (%)	Controls (%)	Cut-off (GPL)
<i>Non-Asian population</i>			
Tuhim et al. [8] (1999)	128/524 (25)	74/1020 (8)	>23
Zielinska et al. [7] (1999)	41/194 (21)	6/100 (6)	>20
D'Olhaberriague et al. [19] (1998)	37/300 (12)	6/149 (4)	>20
<i>Asian population</i>			
Wang et al. [12] (1992)	4/246 (2.3)	–	M+3SD
Kitagawa et al. [13] (1991)	22/250 (8.8)	–	M+3SD
Present study (2005)	12/273 (4.4)	2/181 (1.2)	>20
p value	0.0066*	<0.0001**	

M+3SD = Mean plus 3 standard deviations from normal controls; – = no data.

* Comparing Asians and non-Asians ischemic stroke patients; ** comparing Asians and non-Asians controls.

Discussions

- ▶ Model I: Unadjusted
- ▶ Model II: Adjusted by age and sex
- ▶ Model III: Adjusted by age, sex, smoking, hypertension, diabetes, atrial fibrillation, left ventricular hypertrophy and hyperlipidemia

Table 3. Odds ratios of the logistic regressions for stroke patients and their controls

	Model I*		Model II**		Model III***	
	OR (95%CI)	p value	OR (95%CI)	p value	OR (95%CI)	p value
ACL IgG ≥ 20 vs. <20	4.11 (0.91–18.60)	0.0662	4.01 (0.87–18.37)	0.0739	5.25 (1.06–25.89)	0.0419
ACL IgM ≥ 15 vs. <15	0.94 (0.46–1.92)	0.8707	0.96 (0.47–1.98)	0.9203	1.03 (0.46–2.29)	0.9521

Discussions

- ▶ Studies of stroke victims under 40 years of age have found that the studied antibodies are associated with incident ischemic stroke.
- ▶ Several investigations have provided **conflicting Results** with respect to the significance of elevated aCL levels in older patients.



Discussions

- ▶ Table 4. Prevalence of a higher titer of aCL IgG in non-Asians and Asians

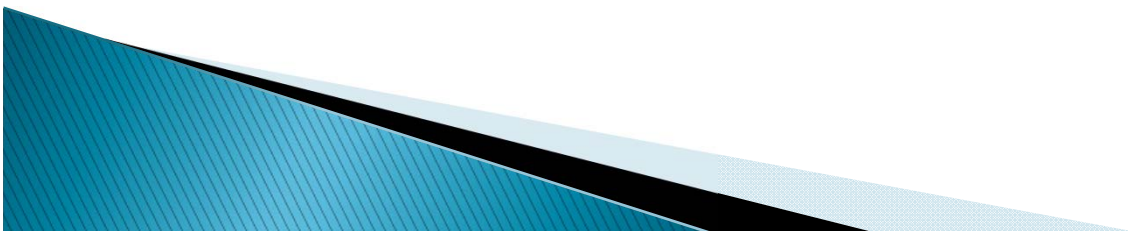
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M+3SD = Mean plus 3 standard deviations from normal controls; – = no data.

* Comparing Asians and non-Asians ischemic stroke patients; ** comparing Asians and non-Asians controls.

Discussions

- ▶ The Framingham study reported that high aCL levels predict the occurrence of an incident of ischemic stroke.
- ▶ The Framingham study adjusted for age, prior CVD, and C-reactive protein.



Discussions

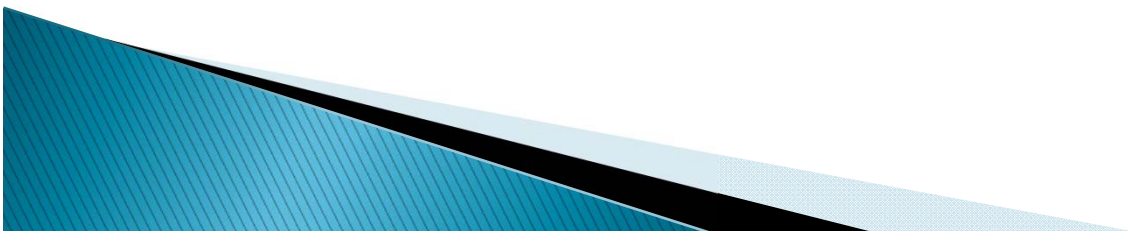
- ▶ Given the fact that multivariate analysis is a more precise estimation than univariate analysis.
- ▶ After adjustment for age, sex, smoking, hypertension, diabetes, atrial fibrillation, left ventricular hypertrophy and hyperlipidemia, ischemic stroke patients aged over 40 years compared to controls. **A fivefold increase** in stroke risk was also observed.

Conclusions

- ▶ The Results of this study suggest that elevated titer of aCL IgG (>20 GPL) is associated with first-ever ischemic stroke in Taiwanese patients aged over 40 years.
- ▶ High positive aCL titer is related to ischemic stroke after adjustment for conventional cerebrovascular risk factors, indicating that it is probably an independent risk factor for ischemic stroke.

Future

- ▶ A separate analysis has demonstrated that aCL is associated with cerebral stroke and myocardial infarction, but not with deep vein thrombosis.



Thank you for your attention!!!



Questions???



Questions???



Questions???

- ▶ 37 李碩堯
- ▶ Q:本篇只有在model III才看到各組間的顯著差異，model I和model II都沒有看到，那為什麼還要把model II放進來呢？
- ▶ A:那是一開始作者的研究順序，原本是要探討性別之間的影響，調整之後發現還是沒有顯著差異，故才進行了第三個model來排除更多risk factor

Questions???

- ▶ 69謝蓁
- ▶ Q:為什麼一開始有提到aCL IgM，可是之後的討論卻只在討論aCL IgG了？
- ▶ A:會把aCL IgM放進來是因為之前別人的實驗都有個別在探討IgM和IgG的isotype，只是本篇文章最後都只發現到aCL IgG比較和疾病有相關性所以到最後就只在探討這部分。其實也和前人的結果符合，就是aCL IgG 的level對於疾病的影響比較aCL IgM來的明顯

Questions???

- ▶ 132李易軒
- ▶ Q:請問第27頁，238是指人數，旁邊為什麼會有百分比呢？
- ▶ A:那個並不是百分比，而是標準差，下面的項目才是百分比。

Questions???

- ▶ 118劉郁欣
- ▶ Q:為什麼cancer 和connective tissue diseases不像其他risk factor一樣等到最後再用logistic regression去掉?
- ▶ A:Cancer 和 connective tissue diseases和其他risk factor 不一樣，他們是直接影響了aCL的濃度，所以既然已經知道他們的直接相關性了就直接排除。

Questions???

- ▶ 老師：覺得本篇論文有什麼可以改進？
- ▶ 士剛：我覺得控制組選得很有爭議，因為沒辦法完全說有這些疾病的患者，並不會影響此次統計的結果，而實驗組，若是能一開始就排除心血管因子，雖然可能有些難度，但是這樣討論就更具意義。
- ▶ 佳駿+冠閔：本篇的IgM和IgG的level是個別探討的，可以深入探討IgM和IgG的level在同一個病人上的不同的組合和疾病的相關性（考慮到IgM 和 IgG之間是有可能會有一些相互關係，也可以探討）

Questions???

- ▶ 135 劉冠宏
- ▶ Q:有沒有可能這樣的做法在調整之後，實際上沒有相關的**risk factor**也變成有相關？
- ▶ A:任何的實驗都有可能出現偽陽性的情況，唯有加大樣本數來解決這個問題了。