行政院國家科學委員會專題研究計畫　成果報告

兒童嚴重急性呼吸道症候群臨床研究

計畫類別：個別型計畫
計畫編號：NSC92-2751-B-002-013-Y
執行期間：92 年 07 月 01 日至 93 年 06 月 30 日
執行單位：國立臺灣大學醫學院小兒科

計畫主持人：黃立民

計畫參與人員：黃立民　邵蓓嵐　高全良　呂俊毅　張鑾英

報告類型：完整報告

處理方式：本計畫可公開查詢

中　華　民　國 93 年 12 月 20 日

中文摘要

目的：嚴重急性呼吸道症候群(Severe acute respiratory syndrome, SARS)是一新興感染症，其致病原為新發現的冠狀病毒(SARS-associated coronavirus)。許多呼吸道病毒的主要宿主均為兒童，然而目前嚴重急性呼吸道症候群的通報病例及研究成果多在成人，對其在兒童與青少年的臨床表徵了解不多。因發燒是兒童相當常見的症狀，其中肺炎又是兒童常見之疾病，因為兒童 SARS 的診斷不易且常易與其餘非典型肺炎如黴漿菌或病毒性肺炎相混淆，為了能使兒童 SARS 之診斷能更加快速及準確，故本計畫將發展成本較低、操作簡便、敏感性及特異性高的抗體偵測方法 – 即 IFA (間接免疫螢光測試)及 ELISA (酵素免疫測試)，以作為 SARS 流行期間快速診斷的依據。在有方便可靠的抗體偵測工具後，本計畫將針對兒童 SARS 研究其清流行病學及臨床特徵，並追蹤其傳染途徑及傳染力。

方法：本研究 IgG 抗體測試採用間接免疫螢光法(IFA)，為偵測此法敏感性及特異性，採 17 位內科 SARS 病患恢復期血清作為陽性對照，204 位兩年前收集之 18 歲以下兒童血清作為陰性對照，測試結果敏感性及特異性均為 100%。本計畫自 2003 年 4 月開始一年內，針對十八歲以下的病患於研究期間於小兒科住院或至急診就診者，收集其血清進行 IgG 測試，以了解 SARS 血清流行病學，對於 IgG 陽性者分析其臨床特性並收集家族成員血清進行研究，以釐清傳染途徑。同時發展操作簡便的酵素免疫法(ELISA)，測試其最佳的反應條件以達最好的敏感性及特異性。

結果：經調整緩衝液成分及反應時間後，使用全病毒抗原之 ELISA 可達 96.4%的敏感性及 100%的特異性。在偵測兒童 SARS 病例方面，自 2003 年 4 月至 10 月，共收集 422 位病患血清，179 位為呼吸道感染，137 位為非呼吸道感染症，101 位為非感染症，共有七位 IgG 陽性或培養及 PCR 陽性，佔所有病患 1.66%，呼吸道病患 2.79%。所有病患臨床症狀與一般肺炎難以區別，無一需要插管呼吸器，且所有皆完全復原。合併感染比率在小兒科病患相當高(4/5)，推測可能無合併感染症狀皆較輕微而不需至大醫院就診。所有病童家族成員血清皆為陰性反應，表示沒有家族成員間的傳播。另外所有血清陽性且診斷肺炎者皆在 2003 年 7 月之前，表示 7 月之後即無新增 SARS 病例。

結論：在 2003 年 SARS 流行期間小兒感染比例低。其症狀不具特異性，雨一般肺炎難以區別。小兒科 SARS 病症較成人輕微且較不具傳染性，合併感染比例高，並未發現有家族內傳播的情形。使用 ELISA 測定 SARS-CoV 抗體是一操作簡便同時具有高敏感性及特異性的檢查，在 SARS 流行期間可作為快速診斷的參考，也可作為調查 SARS 流行病學的工具。

英文摘要

Background and purpose: Severe acute respiratory syndrome (SARS) is an emerging infectious disease caused by a novel SARS-associated coronavirus (SARS-CoV). In contrast to most viral diseases, clinical data about SARS are limited to adults and little about pediatric SARS have been reported. The purpose of this study is to identify the seroepidemiology and clinical features of SARS in pediatric patients. We also tried to clarify the transmission route and infectivity of pediatric SARS patients. Meanwhile, fever and pneumonia are common diseases in children. It is challenging to distinguish SARS from other respiratory tract infections in pediatric populations during the outbreak period. Therefore, we also try to develop the reliable and easy to perform tools for rapid diagnosis of SARS and epidemiological study.

Methods: We collected blood samples from patients younger than 18 years old and admitted to National Taiwan University Hospital pediatric wards or visited pediatric emergency department. Serum IgG to SARS-CoV was tested by indirect immunofluorescent assay (IFA). The sensitivity and specificity of IFA were detected by the 86 sera from documented adult SARS patients and the 204 negative sera collected 2 years ago. Both the sensitivity and the specificity were 100%. Virus culture or detection of viral RNA by PCR from throat or nasopharyngeal swabs was done in some patients. We analyzed the clinical features of the cases with positive antibody response or culture/PCR result and measured the antibodies of family members living in the same household with the index case. We also test the different reagents and reaction intervals to optimize the performance of the IgG ELISA.

Results: After adjusted the buffer contents and the reaction intervals, the IgG ELISA reached the sensitivity of 96.4% and the specificity of 100%. From April 2003 to October 2003, a total of 422 serum samples were collected. Six patients were seropositive for IgG anti-SARS CoV and one excreted SARS CoV in the feces. Among them, 5 patients were experiencing pneumonia. They accounted for 1.66% of all patients and 2.79% of the patients with respiratory tract infections. The clinical features of SARS patients were not specific. None of the SARS patients developed respiratory failure. However, coinfections with other pathogens were common in pediatric SARS patients (4/5). All the family members of the index case had negative antibody response.

Conclusion: Few children were infected during the outbreak period in Taiwan in 2003. Pediatric SARS patients usually ran a benign disease course. The clinical features were not specific to distinguish SARS from pneumonia caused by other pathogens. Coinfection was common. Pediatric SARS patients were less contagious than adult patients. No intra-familial spread was documented. The whole virus antigen

IgG ELISA is easy to perform and with high sensitivity and specificity. It can be used for rapid diagnosis during the SARS outbreak period and for seroepidemiology study.

目錄

一、前言
二、研究目的
三、文獻探討
四、研究方法
五、結果與討論
六、附錄

一、前言

Severe Acute Respiratory Syndrome (SARS), caused by a novel coronavirus, is an emerging infectious disease that first manifested in humans in Guangdong Province, China in November 2002[1]. It has subsequently spread worldwide rapidly. As of May 31, 2003, a cumulative total of 8360 probable cases with 760 deaths had been reported from 28 countries[2]. The majority of patients have been adults aged 25 to 70 years old who were previously healthy[3]. The clinical characteristics and short-term outcomes had been well described in adult patients of SARS. The incubation period for SARS is typically 2 to 7 days, maybe as long as 10 days. Fever, rigors, myalgia, headache and cough are common complaints. Suggestive laboratory features include lymphopenia, thrombocytopenia, elevated liver enzymes, creatine phosphokinase and lactate dehydrogenase levels. In 10%--20% of cases, the respiratory illness is severe enough to require intubation and mechanical ventilation. The case-fatality rate is approximately 9% as accumulated deaths in May 2003.

After the first case identified on March 14 in Taiwan, the cases increased rapidly mainly from the outbreak in hospitals. By May 31, 2003, there were more than 670 probable cases reported and more than 10,000 people quarantined in Taiwan. SARS-CoV is highly contagious within the close contactors and the severity of SARS seems to be variable, ranging from asymptomatic, mild illness to death. Therefore, it is critical to have reliable diagnostic tools to detect the SARS cases.

Rapid identification of SARS-CoV as the etiologic agent of SARS and extensive international collaboration has aided in the development of diagnostic tests. Polymerase chain reaction (PCR) can detect genetic materials of the SARS-CoV in various specimens (blood, stool, respiratory secretions or body tissues). Detectable immune responses begin on day 5 or 6 after the onset of the symptoms. Different types of antibodies (IgM and IgG) appear and change in level during the course of infection. They can be undetectable at the early stage of infection. IgG usually remains detectable after resolution of the illness. IFA (Immunofluorescence Assay) has been developed for detecting IgM antibodies in serum of SARS patients, which yields positive results after about day 10 of illness. This test format is also used to test for IgG. This is a reliable test requiring the use of fixed SARS virus on an immunofluorescence microscope. Positive antibody test results indicate a previous infection with SARS-CoV. Seroconversion from negative to positive or a four-fold rise in antibody titre from acute to convalescent serum indicates recent infection. No detection of antibody after 21 days from onset of illness seems to indicate that no infection with SARS-CoV took place.

Only few cases of SARS have been reported among young population. By June 1, 2003, Taiwan CDC and US CDC reported 46 (6.8%), 13 (20%) probable cases in

those under 18 years old[4,5]. In April 2003, Hon et al[6] reported the first ten children with SARS managed during the early phase of the epidemic in Hong Kong. All the children had been in close contact with infected adults. Persistent fever, cough, progressive radiographic changes of chest and lymphopenia were noted in all patients. Their study also showed that the clinical course was milder and shorter, and the radiological changes were milder and generally resolved more quickly in young children than in the teenagers. Lymphopenia was severer among teenagers and the interpretation of results must take into account to the patients' ages.

In addition to the household contact with SARS patients, local transmission in the community, such as in Taipei city, and travel to the affected areas also put children and teenagers at risk of contacting SARS-CoV. Furthermore, respiratory tract infections are the most frequently occurring illness in childhood. Early symptoms of SARS are nonspecific and are indistinguishable with other common viral infections. The clinical characteristics of pediatric SARS remain undefined. It is a great challenge to diagnose SARS in young population when contact history becoming difficult to access in a community with local transmission. Therefore, sensitive diagnostic tests along with clinical characteristics and epidemiological evidences help to the diagnosis of SARS in pediatric population.

二、研究目的

To have a reliable diagnostic tool for SARS-CoV infection is an urgent need. The current available tests, including virus culture, RT-PCR for viral RNA, and IFA, are insensitive, time-consuming, or labor-intensive. This makes large-scale epidemiological screening impractical. Therefore, this project will develop the enzyme-linked immunoabsorbent assay for SARS-CoV antibodies with high sensitivity and specificity. The ELISA will be easier to perform, less labor-intensive than the IFA.

As we know, the major hosts of many respiratory viruses are children, but most data about SARS so far and the majority of reported cases are adult. The data of clinical presentation and epidemiological prevalence in teenage group and children are lacking. Whether the children and adolescent are more resistant to infection or the clinical symptoms are subtler is still unknown. Besides, whether they are highly contagious as adult patient still needs further study. Therefore, this project will aim at the children and teenager below eighteen years old. The results will delineate the clinical course and epidemiology of SARS in children and adolescents, and it also provides the objective early indicator of SARS diagnosis in children and adolescents. This will also define the role of childhood and adolescents in the transmission of SARS.

After prospectively surveying all the pneumonia patients, we can estimate the percentage of SARS as the cause of pneumonia in young children and teenagers. We can also identify the subclinical and asymptomatic infection of SARS-associated virus among young children and teenagers by serologic survey.

三、參考文獻

1. World Health Organization. SARS epidemiology to date. April 11. Available at http://www.who.int/csr/sars/epi2003_04_11/en/
2. World Health Organization. Cumulative number of reported cases of severe acute respiratory syndrome (SARS). Available at http://www.who.int/csr/sars/country/2003_05_31/en
3. CDC. Preliminary Clinical Description of Severe Acute Respiratory Syndrome.MMWR 2003,52(12) 255-256
4. CDC. Update: Severe Acute Respiratory Syndrome --- United States, May 30, 2003 MMWR 2003,52(21) 500-501
5. 衛生署疾病管制局 http://www.cdc.gov.tw/sars/4 國內 SARS 相關資訊/台灣 SARS 疫情現況.htm
6. K L E Hon, C W Leung, W T F Cheng, Clinical presentations and outcome of severe acute respiratory syndrome in children The Lancet (361) Issue: 9370 May 17, 2003 1701-1703

四、研究方法

Study subjects

Two groups of subjects, who were under 18 years old, will be included. Group 1 includes those who visited our hospital due to fever or respiratory infection during the epidemics in our country in Apr and Jun 2003. Throat swab were obtained for virus culture and RT-PCR for viral RNA. Group 2 are those who admitted to our hospital among Apr 2003 to Oct 2003. Serum samples were obtained for SARS-CoV antibodies assay.

The medical charts of suspect/probable SARS cases will be reviewed. The epidemiological and clinical features will be analyzed. The convalescent sera will be collected after 28 days of onset. The sera of the family members living in the same household will also be collected to clarify the intra-familial transmission.

Case definitions

SARS case definitions have been developed and revised by WHO. A working definition modified by Taiwan CDC is used. A contact is a person who may be at greater risk of developing SARS because of exposure to a suspect or probable case of SARS. Information to date suggests that risky exposures include having cared for, lived with, or having had direct contact with the respiratory secretions, body fluids and/or excretion (e.g. faeces) of a suspect or probable cases of SARS. Close contact includes those having cared for, lived with, or had direct contact with respiratory secretions or body fluids of a suspect or probable case of SARS.

Pneumonia is defined as fever, respiratory symptoms such as cough, tachypnea, dyspnea, or hypoxia, and changes in CxR.

Reverse transcription-polymerase chain reactions

RNA extracts from throat swabs are prepared from 100 μl of each specimen with the automated Roche extraction system (Roche). Oligonucleotide primers used for amplifying and sequencing of the SARS-related coronavirus are designed from alignments of open reading frame 1b of the coronavirus polymerase gene sequences obtained from GenBank. Three SARS-specific primers, Cor-p-F2 (+) 5'CTAACATGCTTAGGATAATGG3', Cor-p-F3 (+) 5'GCCTCTCTTGTTCTTGCTCGC3', and Cor-p-R1 (–) 5'CAGGTAAGCGTAAAACTCATC3', will be used to test patient specimens in this study. All specimens were tested for human glyceraldehyde-3-phosphate dehydrogenase to confirm RNA integrity and control for RT-PCR inhibition.
One primer for each set is 5'-end-labeled with fluorescent dye 6-carboxyfluorescein (6-FAM) to facilitate GeneScan analysis. One-step amplification reactions are performed with the Access RT-PCR System (Promega) as previously described. Positive and negative RT-PCR controls, containing standardized viral RNA extracts

and nuclease-free water, are included in each run. Amplified 6-FAM–labeled products will be analyzed by capillary electrophoresis on an ABI 3100 Prism Genetic Analyzer with GeneScan software (version 3.1.2). Specimens are considered positive for SARS-associated coronavirus if the amplification products are within 1 nucleotide of the expected product size (368 nucleotides for Cor-p-F2 or Cor-p-R1 and 348 nucleotides for Cor-p-F3 or Cor-p-R1) for both specific primer sets, as confirmed by a second PCR reaction from another aliquot of RNA extract in a separate laboratory. Where DNA yield was sufficient, the amplified products will also be sequenced.

Indirect immunofluorescence assay (IFA)

The Vero E6 cells, latently infected with SARS virus, are used in this assay. Thereafter, the cells are fixed onto glass slides by cold acetone and blocked by incubation with PBS containing 5% BSA for 30 min in a humidified chamber. The cell culture and slides fixation will be done in the P3 lab. The slide is then overlaid with diluted patient serum in blocking solution (5% BSA in PBS) and incubated for 1 hr. For IgG test, the sera are diluted in 1:25 and 1:50. For IgM and IgA test, the sera are diluted in 1:20. Unbound serum is washed away with PBS for 10 min repeated 3 times. The secondary antibody, gout anti-human IgG_{123}, diluted 1:2000 in blocking solution is then added and incubated for 1 hour. Washing with PBS for 3 times is performed again followed by adding FITC-conjugated goat anti-mouse IgG diluted 1:4000 in blocking solution and incubated for another hour. After the final wash, the slide is observed under immunofluorescence microscope with mounting medium (PBS:glycerol=1:9). The antibody titer is defined as positive when positive fluorescence observed in at least 5% of cells. Several serum samples derived from SARS patients are tested positive and served as positive controls for the serologic assay.

Enzyme-linked immunosorbent assay

Preparation and identification of whole-virus antigen for ELISA

SARS-CoV isolate (TW1) was propagated in Vero E6 cells, in MEM (JRH, Lenexa, USA)-medium supplemented with 2% fetal calf serum (FCS). Viruses were harvested from culture supernatant at 48 hours after inoculation when more than 90% of cells showed cytopathogenic effects. After centrifugation (600 X g for 10 min) and discarding the pellets containing exfoliated cells, the supernatants were heated at 56°C for one hour to inactivate SARS-CoV and were filtered through 0.2 µM pores (Sartorius, Goettingen, Germany) to dispose of bacteria. The supernatants were suspended in 20% polyethylene glycol (Sigma, St. Louis, USA) and centrifuged again at 8,000 X g for 4 hours at 4 ^{o}C. The pellets containing viruses were resuspended in PBS buffer and purified by 35-80% sucrose gradients centrifugation (8,000 X g for 16 hours at 4 ^{o}C). Viral fraction was washed with PBS and then centrifuged at 8,000 X g

for 4 hours at 4 ^{o}C. The pellets contained the purified whole cell viral antigens. The concentration of purified viral proteins was measured using a Bio-Rad protein assay kit (Bio-Rad, Hercules, USA).

The purified whole virus antigen was subjected to polyacylamide electrophoresis and stained with Coomassie blue and Western blotting analysis using SARS patients' sera to understand the proteins contained in this preparation.

IgG ELISA using SARS-CoV whole virus antigen

Five μg of purified viral protein was diluted in 200 μl 50 mM Na_2CO_3 (pH 9.5) and was used to coat the wells of microtiter plates at 4 ^{o}C overnight. The plates were then blocked with 380 μl/well blocking buffer [5% FCS in Superblock, (PIERCE, Rockford, USA)] at room temperature for 1 hour. The sera were pre-treated at 56 ^{o}C for 30 minutes and then diluted at 1:50 in blocking buffer. Two hundred μl-diluted sera were added to each well and incubated at room temperature for 30 minutes. The plates were then washed five times in washing buffer containing PBS and 0.5% Tween 20. Thereafter, 200 μl of goat anti-human IgG antibody (Chemicon, Temecula, USA) diluted at 1:80,000 in blocking buffer was added to each well and incubated at room temperature for 30 minutes. The plates were washed five times in washing buffer again. The specific anti-SARS-CoV IgG was detected by adding 100 μl of TMB (KPL, Gaithersburg, USA). Absorbance was measured at 450nm after adding 100 μl of H_2SO_4 solution (1N).

Cut-off value determination

We tested sera collected from patients visiting National Taiwan University Hospital for diseases other than SARS to determine the cut-off O.D. value for the IgG ELISA. None of the patients whose sera were used for cut-off value determination had a prior contact history with SARS patients. All the sera had been confirmed to be seronegative for SARS-CoV by immunofluorescence assay (IFA) at the dilution of 1:25. The mean O.D. value of the sera plus 3 standard deviations was calculated as the cut-off O.D. value of the IgG ELISA.

ELISA diagnostic sensitivity and specificity

To determine the sensitivity and specificity of the ELISA, true positive and true negative control sera were defined and tested. True positive control sera were those collected from patients of SARS and tested positive by IFA. Clinical SARS patients were diagnosed according to WHO criteria [16]. True negative control sera were those collected in 2000 and 2001 before SARS had been first reported and tested negative by IFA.

五、結果與討論

1. 本計畫的 IgG、IgM、IgA 抗體偵測採用 IFA 的方法，為偵測此法敏感性及特異性, 採集 17 位內科 SARS 病患恢復期不同時間點之血清共計 54 管作為陽性對照, 200 管採集於 1999 至 2000 年之 18 歲以下健康兒童之血清作為陰性對照，測試結果 54 管陽性對照血清之 IgG，IgM 均為陽性，IgA 出現陽轉時間雖不一定，但所有病患最終皆有陽轉。而 200 管陰性對照血清測試均為陰性，顯示 IFA 抗體測試之敏感性及特異性均為 100%。雖然 IFA 具高敏感性及特異性, 但在操作及判讀結果上耗時費力, 將其推廣使用至大規模的篩檢病患有實行上的困難。

所以同時亦發展了 ELISA，作為更為快速，操作更簡便之抗體測試方式。首先我們先發展 IgG 之 ELISA，發展初期結果之背景值皆過高，之後在調整 Blocking Buffer 及 Washing Buffer 的成分後，成功的降低非特異性反應。以此法測試 56 管陽性對照及 204 管陰性對照血清，結果所有陽性對照中五十三管為陽性，一管為陰性反應，所有陰性對照皆為陰性反應，其中敏感性為 96.4% 而特異性為 100%。顯示此法與上述 IFA 同樣有相當高之敏感性及特異性，且與 IFA 有相當高之相關性。使用 ELISA 測定 SARS-CoV 抗體是一操作簡便同時具有高敏感性及特異性的檢查，在 SARS 流行期間可作為快速診斷的參考，也可作為調查 SARS 流行病學的工具。ELISA 的成功研發已完成論文寫作並已被接受，即將刊載於 Journal of biomedical science。

2. 對 2003 年 4 月至 6 月 SARS 流行高峰期前來求診之兒童，採集喉頭拭子共計 134 管，一方面進行病毒培養，一方面抽取其 RNA 進行 RT-PCR，測試病毒核酸。結果只有一位兒童病毒培養及 RT-PCR 均為陽性反應, 對照其抗體測試, 由於只有偵測到該位病童發病急性期及 21 天後之血清，結果均為陰性，但此與 WHO 規定須追蹤至 28 天之排除條款不合，故無法確定此位病童是否確實無抗體陽轉。

3. 收集自 2003 年 4 月至 10 月間共計 422 位前來台大住院或至急診求診之 18 歲以下病童之血清，其中 179 位為呼吸道感染病人， 137 位為非呼吸道之感染症病人，101 位為非感染症病人， 5 位病人之診斷未明。測試結果 IgA 全部皆為陰性反應，IgM 在急性期住院中有 17 位病患為陽性反應，其中有 6 位追蹤至 28 天後 IgG 抗體結果並未陽轉，顯示兒童 IgM 之 IFA 測試有相當部份病人會因與其他兒童常見感染症有交叉反應而產生偽陽性。IgG 部分有 6 位病人為陽性反應, 佔所有病人 1.42%, 其中有 4 位肺炎病人之 IgG 測試為陽性反應, 佔肺炎病人之 2.23%。

關於 IgM 之偽陽性部分，可能因 IgM 分子較大，由於一開始採用 Acetone 作固定，無法使 IgM 分子進入受感染之細胞中，產生較具特異性之螢光反應型態，而與非特異性結合的偽陽性較難以區分。之後計畫測試使用 Acetone 加 Methanol 作固定，可使細胞膜上孔洞變大，而使 IgM 較容易進入，可加強與偽陽性的區別。

在 4 位 IgG 陽性之肺炎病人，亦即當次住院為 SARS 急性感染，皆發生在 2003 年 7 月以前，顯示 7 月之後已無新增 SARS 病例。2 位 IgG 陽性之非肺炎病人可能為流行期間受感染但症狀不顯著，故當時並未求醫，而 IgG 抗體持續至測試時。4 位 SARS 急性感染病人均有做喉頭拭子，結果病毒培養及核酸 RT-PCR 測試皆為陰性，可能因為兒童感染後 viral load 均不高，故偵測不到。

4. 總共 7 位 SARS 急性感染或曾感染病人在接觸史方面，有 2 位從國外疫區回來後發病，有 2 位曾接觸疫區回來之成人，而成人本身並無症狀，有一位住在疑似有 SARS 感染病患之社區且家中有曾到過疫區之成人，但成人本身並無症狀，有 2 位則完全沒有接觸史。在 5 位 SARS 急性感染之病人（4 位抗體陽性及 1 位 PCR 陽性）年齡為 4 歲至 13 歲，男女比為 2 比 3，3 位有明顯淋巴球低下（淋巴球數小於 1000/**μL**），大部分（4/5）有明顯 CRP 上升（4.96 至 13.73mg/dl），所有病人 CPK 均無明顯上升，有 3 位曾做 LDH 測試，3 位皆有明顯上升（494-1004 **U/L**）。胸部 X 光有 3 位顯示有單一或多處 Patch，2 位則以 infiltration 為主。有 1 位 4 歲女童因呼吸急促血氧低下需氧氣治療，而此臨床較嚴重之女童有最明顯淋巴球低下及最高之 LDH 值。合併感染可見於 4 位病童（1 位血清 Chlamydia 抗體陽性，1 位痰液細胞融合病毒抗原陽性，1 位尿液肺炎雙球菌抗原陽性，另一位同時有尿液肺炎雙球菌抗原陽性及痰液 Chlamydia 抗原陽性）。發燒天數從 2 天至 7 天，全部皆有咳嗽症狀，2 位有肌肉酸痛症狀，3 位有頭痛症狀，1 位有腸胃不適症狀。所有病患皆不需插管呼吸器治療，且所有病患皆完全康復。

在 7 位 SARS 急性感染或曾感染病人中，只有 2 位從國外疫區移入，其餘 5 位並無明顯與 SARS 病患接觸史，定義上可算是社區感染，但篩檢 422 位病患只有 5 位為社區感染 SARS，比例相當低。兒童 SARS 病人臨床表現與其他常見於兒童之非典型或細菌性肺炎難以區分，並不具特異性，故以臨床症狀診斷 SARS 有其困難。本計畫發現合併感染比率相當高，推測若無合併感染，兒童 SARS 症狀可能相當輕微，而不至於至大醫院求診。總體而言兒童 SARS 症狀一般比成人輕微，且均可完全康復，症狀與其他兒童常見呼吸道感染難以區分，但因社區感染比率低，故臨床上對於 SARS 診斷，接觸史仍是相當重要參考依據。

5. 在 7 位 SARS 急性感染或曾感染病人中，有 3 位完整追蹤至 28 天且有完整家庭成員血清者，28 天後追蹤血清，除了病童本身外，所有家族成員皆為陰性。有一位培養及 PCR 陽性之病童只追蹤至 21 天血清，但其血清呈陰性，其父母有追蹤至 28 天其結果均為陰性。兒童 SARS 病患幾乎均為家庭中唯一受感染者，顯示另有其他感染源，同時均無家庭傳染情況，顯示兒童 SARS 傳染力不如成人強。

結論及建議：在 2003 年 SARS 流行期間小兒感染比例低。其症狀不具特異性，與一般肺炎難以區別。小兒科 SARS 病症較成人輕微且較不具傳染性，合併感染比例高，並未發現有家族內傳播的情形。使用 ELISA 測定 SARS-CoV 抗體是一

操作簡便同時具有高敏感性及特異性的檢查, 在 SARS 流行期間可作為快速診斷的參考，也可作為調查 SARS 流行病學的工具。

六、計畫成果自評部份

This project elucidate the epidemiology of 2003 SARS epidemic in Taiwanese children. Our results were consistent with what we have observed clinically. The project has been modified slighyly because of the prolonged closure of p3 lab in our medical campus due to tightened regulation on all the p3 labs in Taiwan. ELISA project was added instead.

七、附錄，並請註明發表刊物名稱、卷期及出版日期

1. Chang LY, Huang FY, Wu YC, Su IJ, Chiu NC, Chen KT, Wu HS, Lin TH, Peng SF, Kao CL, Lee CY, **Huang LM***. Childhood severe acute respiratory syndrome in Taiwan and how to differentiate it from childhood influenza infection. Archives Pediatr Adolesc Med 2004; 158: 1037-1042. (* Corresponding author) (original article) (SCI journal)
2. Shao PL, Hsueh PR, Chang LY, Lu CY, Kao CL, Chiang YP, Huang HY, Huang FY, Lee CY, **Huang LM***. Development of Immunoglobulin G Enzyme-linked Immunosorbent Assay for the Serodiagnosis of Severe Acute Respiratory Syndrome. J Biomed Sci accepted (* Corresponding author) (original article) (SCI journal)
3. Chen YC, Huang LM, Chan CC, Chang SC, Su CP, Sheng WH, Chang SC, Chang YY, Chen ML, Hung CC, Chen WJ, Lin FY, Lee YT. Potential role of the environment in the transmission of severe acute respiratory syndrome in a hospital Emergency room. EID 2004; 10: 782-788. (SCI journal) (original article).