ประโยชน์ของการทดสอบเซลล์เยื่อจมูกในการวินิจฉัยผู้ป่วยโรคจมูกอักเสบจากภูมิแพ้ในเด็ก

รณชัย วิริยะทวีกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาการพัฒนาสุขภาพ หลักสูตรการพัฒนาสุขภาพ
คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2543
ISBN 974-347-164-2

THE USEFULNESS OF NASAL CYTOLOGY TEST IN THE DIAGNOSIS OF ALLERGIC RHINITIS IN CHILDREN

Ronnachai Viriyataveekul

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Health Development

Program of Health Development

Faculty of Medicine

Chulalongkorn University

Academic Year 2000

ISBN 974-347-164-2

Title	**: The usefulness of nasal cytology test in the diagnosis of allergic rhinitis in children**
By	**: Ronnachai Viriyataveekul, M.D.**
Field of Study	**: Health Development**
Thesis Advisor	**: Associate Professor Sompop Limpongsanurak, M.D.**
Thesis Co-advisor	**: Professor Visanu Thamlikitkul, M.D., M.Sc.**

.....

Accepted by Faculty of Medicine, Chulalongkorn University in partial fulfillment of the requirements for Master's Degree

..... Dean of Faculty of Medicine

(Professor Pirom Kamol-ratanakul, M.D., M.Sc.)

..... Chairman

(Professor Chitr Sitthi-amorn M.D., Ph.D.)

..... Thesis Advisor

(Associated Professor Sompop Limpongsanurak, M.D.)

..... Thesis Co-advisor

(Professor Visanu Thamlikitkul, M.D., M.Sc.)

..... Member

(Sutthipol Udompanthurak, M.Sc.)

รณชัย วิริยะทวีกุล: ประโยชน์ของการทดสอบเซลล์เยื่อจมูกในการวินิจฉัยผู้ป่วยโรคจมูกอักเสบจากภูมิแพ้ในเด็ก (THE USEFULNESS OF NASAL CYTOLOGY TEST IN THE DIAGNOSIS OF ALLERGIC RHINITIS IN CHILDREN) อาจารย์ที่ปรึกษา: รศ.นพ.สมภพ ลิ้มพงศานุรักษ์ พบ., วว., (สูติ-นรีเวชวิทยา) อาจารย์ที่ปรึกษา ศ.นพ.วิษณุ ธรรมลิขิตกุล พ.บ., วว. (อายุรศาสตร์) .69 หน้า ISBN 974-347-164-2

เด็กไทยได้รับการวินิจฉัยว่าเป็นโรคจมูกอักเสบจากภูมิแพ้โดยไม่มีการทดสอบใด ยืนยัน มักจะได้รับการรักษาที่ไม่เหมาะสม การทดสอบเซลล์เยื่อจมูกเป็นวิธีที่ทำได้ง่าย อาจจะช่วยกุมารแพทย์ในการยืนยันการวินิจฉัยโรคจมูกอักเสบจากภูมิแพ้ได้ วัตถุประสงค์ของการศึกษานี้เพื่อประเมินการทดสอบเซลล์เยื่อจมูกด้วยค่าความถูกต้องแม่นยำ, ค่าการทำนายโรค และความน่าจะเป็นในการวินิจฉัยโรคจมูกอักเสบจากภูมิแพ้

ได้รวบรวมเด็กชายจำนวน 83 ราย และเด็กหญิง จำนวน 41 ราย ซึ่งมีอาการของน้ำมูกมาประมาณ 2 สัปดาห์ หรือนานกว่านั้น มาทำการทดสอบสะกิดผิวหนังด้วยสารก่อภูมิแพ้ชนิดสูดดมที่พบบ่อย และตรวจเยื่อจมูก อายุของผู้เข้าร่วมโครงการมีตั้งแต่ 29 เดือน ถึง 135 เดือน ค่ามัธยฐาน(median)อยู่ที่ 75 เดือน, ในจำนวนผู้เข้าร่วมโครงการทั้งหมด 124 ราย มีผู้ป่วยได้รับการวินิจฉัยเป็นโรคจมูกอักเสบจากภูมิแพ้ 70 ราย, ค่าความไว, ความจำเพาะ ค่าการทำนายเป็นโรคเมื่อการทดสอบเป็นบวก และค่าการทำนายไม่เป็นโรคเมื่อการทดสอบเป็นลบ ที่ได้ คือ 80%, 90.74%, 91.80% และ 77.78% ตามลำดับ

สรุป การทดสอบเซลล์เยื่อจมูกมีค่าความถูกต้องที่ดี และมีประโยชน์ในการช่วยยืนยันการวินิจฉัยโรคจมูกอักเสบจากภูมิแพ้

ภาควิชา การพัฒนาสุขภาพ ลายมือชื่อนิสิต.....

สาขาวิชา การพัฒนาสุขภาพ ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2543 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4275388230 MAJOR HEALTH DEVELOPMENT

Key word:

Nasal Cytology, Diagnostic Test, Allergic Rhinitis

Ronnachai Viriyataveekul : The usefulness of nasal cytology test in the diagnosis of allergic rhinitis in children. Thesis advisor : Associate professor Sompop Limpongsanurak M.D., M.P.H. (Clinical Epidemiology) Thesis Co-Advisor: Professor Visanu Thamlikitkul M.D., M.Sc. 69 pp. ISBN 974-347-164-2

Some children, who are diagnosed as allergic rhinitis without any laboratory test to confirm, are often treated with inappropriate medications. Nasal Cytology test, which is inexpensive and easy to perform, may help general pediatricians to confirm their diagnosis of allergic rhinitis. The aim of this study was to evaluate the accuracy, predictive values and likelihood ratio of nasal cytology test in the diagnosis of allergic rhinitis.

Eighty three boys and forty one girls, who had nasal symptoms for 2 or more weeks, were recruited to perform skin prick tests with common inhalant allergens and nasal cytology tests. Their ages ranged from 29 months to 135 months and a median of 75 months. Of the 124 subjects, 70 were diagnosed as allergic rhinitis, the sensitivity, specificity, positive predictive and negative predictive values of nasal cytology test (combined positive eosinophil or basophil) were 80%, 90.74%, 91.80% and 77.78% respectively.

The conclusion is that nasal cytology test had good accuracy and is useful to confirm the diagnosis of allergic rhinitis.

Department Health Development Student's signature……

Field of study Health Development Advisor's signature……

Academic year 2000 Co-advisor's signature……

Acknowledgement

Firsty, I would like to thank Associate Professor Benjawan Rungpitarungsri for allowing me to participate in this program, also to Associate Professor Sompop Limpongsanurak for his wise critiscism. I am sure this thesis could not have been completed without the encouragement, valuable advice and quidance of Professor Visanu Thamlikitkul.

I would also like to express my sincere thanks to my colleages in the Department of Clinical Pathology in Siriraj Hospital for their unwaving support especially Associate Professor Nisarat Opartkiattikul, Naravat Poungvarin, Kanit Reesukumal.

For their valuable assistance, I would like to express my gratitude to the staff of Clinical Epidemiology Unit of Faculty of Medicine, Chulalongkorn University.

Last but not least my thanks must go to Faculty of Medicine, Siriraj Hospital, Mahidol University for their funding support.

CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure **Page**

CHAPTER 1

BACKGROUND AND RATIONALE

Rhinitis, allergic and non-allergic, is the most common chronic illness affecting children. Adolescents (age 12-17 years) with rhinitis are certainly bothered by a stuffy/blocked nose, a runny nose, sneezing and problems with concentration, particularly with schoolwork. Younger children (aged 6-12 years) present a slightly different picture. They are certainly bothered by their symptoms and the practical problems of carrying tissues and taking medication. The parents were more bothered by their child's rhinitis than the child. Because rhinitis in the absence of one of its complications is not associated with mortality or hospitalizations, many clinicians tend to trivialize the child with chronic rhinitis. But for the children or their parents, chronic rhinitis is not a trivial disease.

Rhinitis is classified as infectious rhinitis, allergic rhinitis and nonallergic, non infectious rhinitis. In pediatric patients, both infectious and allergic rhinitis are common.

Infectious rhinitis, which is typically viral, is often referred to as a “common cold” or upper respiratory infection (URI). Such infectious rhinitis is much more common in children than adults. Preschool children, approximately 6 up to 12 times a year, suffer from an URI; the incidence decreases with age and averages 2 to 3 common colds a year in adolescents and adults and children with asthma is likely to have more than that figure. Rhinoviruses are the most important virus type in school-age children and adults, while respiratory syncytial virus is important in pre-school-age children and infants. Several studies have shown elevated incidence of mild acute respiratory tract illness among children in day care as compared with home-reared children and this effect occurs primarily in younger children. However, there is virtually no evidence that children in day care have increased rates of lower respiratory illness. In a recent telephone survey the duration and frequency of the complications of URI were studied in children aged 1-3 years. URIs were found to last longer in children aged 1-2 years in daycare setting (8.9 days) than in children in home care or in small care groups (6.8 days). The percentage of URI that lasted more than 15 days ranged from 6.5% for children 1-3 years of age in home care to 13.1% for the same aged children in daycare, perhaps suggesting the complication of sinusitis or recurrent viral infection.

Allergic rhinitis is a clinical condition, associated with an excessive generation of specific IgE. It is characterized by the anterior nasal symptoms of pruritus, sneeze, discharge, and stuffiness, and in chronic or severe disease, there is often an associated loss of sense of smell and inability to taste. For aeroallergen sensitivity the interaction between the environmental allergen and the specific IgE, bound to high-affinity receptors ($FcER_I$) on nasal mucosal mast cells, gives rise both to symptom expression, through release of preformed and newly generated mediators, and to the development of nasal mucosal inflammation, through release of cytokines. This mucosal inflammatory process is associated with endothelial cell activation and the tissue accumulation of eosinophils from the circulation, along with the epithelial localization of both mast cells and eosinophils.

Mast cells are constitutive cells of the normal nasal mucosa but are not found superficially within the airway epithelium. Immunohisto-chemical staining of nasal biopsies with monoclonal antibodies against mast cell tryptase identifies an increase in mast cells within the airway epithelium in both seasonal and perennial rhinitis, in comparison with biopsy findings in nonatopic, nonrhinitic subjects. Cross-linking of IgE on the surface of mast cells by allergen leads to a series of intracellular events culminating in the release of preformed

mediators from mast cells (histamine, tryptase, haparin) and the generation of lipid mediators, including prostaglandin D_2 (PGD_2) and the sulfidopeptide leukotrienes, leukotriene (LT) C_4 and its metabolites LTD_4 and LTE_4. These released mediators induce the nasal symptoms of itch, sneeze, discharge, and blockage, through interactions with receptors present on both neural and vascular elements within the nasal mucosa.

In addition to the effects of acute mast cell degranulation on immediate symptom generation, mast cell degranulation will contribute to the eosinophilic mucosal inflammation that is evident in rhinitis.

Eosinophils are not a normal cellular constituent of the nose. Immunohistochemical staining of nasal mucosal biopsies identifies that eosinophils are evident in nasal mucosal biopsies within the submucosa and epithelium in active rhinitis and their recovery is increased in nasal smear specimens.

Basophils are evident in nasal smear samples in allergic rhinitis and can be demonstrated to increase in a symptomatic seasonal rhinitis patients 24 hr following nasal allergen challenge. Basophils, which, like mast cells, possess high-affinity IgE receptors, are also derived from CD34 + progenitor cells in the bone marrow. An increase in circulating basophils is described in rhinitis consistent with stimulation of these progenitor cells.

Upper respiratory tract infection is often observed in children. Some have symptoms of rhinitis for longer than 2 weeks or recurrent problems. There are several causes which prolong nasal symptoms of URI for longer than 2 weeks e.g. sinusitis, allergic rhinitis, recurrent viral infection and adenoid hypertrophy etc. These patients are often misdiagnosed by clinicians e.g. the patients with allergic rhinitis are diagnosed to suffer from bacterial sinusitis or the patients with recurrent virus induced rhinitis are diagnosed as allergic rhinitis. The tools that general practitioners or pediatricians utilize for diagnosis of allergic rhinitis are patient history and physical examination. The study, which compared case histories alone with provocation tests have shown the sensitivity of 73% and specificity of 67% and the prevalence of allergic rhinitis in Thai children is about 40 %. Therefore, if a patient is diagnosed as allergic rhinitis, the probability of "correct diagnosis is about 47% which is not high enough to change treatment. Another study, which was conducted in children followed from birth to 6 years old, showed the sensitivity and specificity of 80% and 41% respectively. The positive predictive value in this study is 48 % that is too low to trust only the history for diagnosis of allergic rhinitis. Some pediatric patients with allergic rhinitis who do not response to the treatment with antihistamine and nasal decongestant should be further considered nasal corticosteroids. The most important

adverse effect of nasal corticosteroids which should be concerned is about bone growth suppression. Therefore, more evidence is needed to make a decision what treatment should be given to these patients, instead of trusting only history . Nasal cytology is a simple and inexpensive laboratory test when compared with skin prick test that is harmful and manually dexterous. For diagnosis of allergic rhinitis, it is questionable if nasal cytology is appropriate and useful for this purpose or not.

CHAPTER 2

LITERATURE REVIEW

The results of nasal cytology test depend on several factors, one of which is the technique. Various techniques have been used in each step: obtaining, processing, evaluating, and interpreting nasal cytology specimens.

A. Sampling Methods

The selection of a sampling method depends on the requirements of the specimen. Considerations include the age of the patient, the need for repeated sampling, the site, depth, and thickness of the nasal mucosa, and the requirement of simultaneous biochemical studies.

Blown Secretions

In this method, secretions in the nasal airways are blown onto wax paper or a plastic wrap and then placed onto a glass slide. The cells originate from the secretions and may thus reflect a different population from that collected from the epithelium. A disadvantage is

that many children and patients with some nasal disorders cannot produce an adequate secretion specimen.

Smears Taken with Cotton Wool Swabs

This is a simple procedure for obtaining cells the adherent secretions and epithelial layer. As with blown specimens, the cell count varies considerably. However, this method can be used to determine the presence or absence of a specific cell population in terms of a relative proportion .

Imprints

These involve the use of thin plastic strips painted with 1% albumin to produce a sticky surface. The strips are gently pressed onto the mucosal surface, usually the septum. A reasonably good cell yield is obtained and the number of specific cell types counted. Disadvantages of this method include the need to be manually dexterous and the presence of a considerable quantity of secretions on the strip .

Brush Method

This technique employs small plastic-coated, steel-wire brushes with nylon bristles. The brush is placed between the septum and

inferior turbinate and rotated while being removed. The total number of cells can be estimated, as the volume in which the cells are suspended is known. The cells obtained include those from secretions and those from the epithelial surface layer. Local discomfort is a disadvantage.

Nasal Scraping

A nasal specimen can be easily obtained by scraping the surface in the middle third of the inferior turbinate with a plastic curette. The cells harvested from the epithelial lining are well preserved and permit differential evaluation of specific cell elements. Advantages of this method include specificity of sampling site, minimal trauma with no need for anesthesia, ease of repetition, and adequacy of specimens at any age in all nasal conditions. Cell samples can also be used for rapid viral and bacterial diagnostic studies as well as biochemical analysis. The main disadvantage is that the superficial quality of the specimen dose not permit evaluation of changes in the deeper mucosal layers.

The sampling methods of nose blowing and mucosal scraping have been compared in several studies in adults and children. In adults with allergic rhinitis, a specimen adequate for microscopic grading could be obtained 100% of the time by the scraped technique, but in only 60-66% of the patients by the blown method. In

children and in patients with rhinopathies not characterized by an increase in anterior discharge, the yield of evaluable specimens is even lower with the nose-blowing technique. There was no difference between the two techniques as to the presence of neutrophils, and the concordance for the presence of eosinophils was fair (67%). However, eosinophils were found more often in the nasal-scraping sample when they were absent in the blown secretion than vice versa (28% vs.5%). Basophils/mast cells were much more frequently noted in the scrapings than in the blown specimens (70% vs.4%).

Nasal Lavage

This is performed by introducing saline solution in each nasal cavity with the patient's head bent backward during closure of the soft palate. The volume of the return lavage fluid is known and the total number of cells harvested can be calculated. The cells are counted in a hemocytometer and the cell differential determined on a cytospin slide.

The cytological findings from nasal mucosal scrapings and nasal lavages have been studied to examine their correlation. In patients with allergic rhinitis challenged with relevant allergens, scrapings and lavages correlated for increased eosinophil number and percentage and for increased neutrophil count and percentage. In nonallergic patients or allergic rhinitis patients provoked by an irrelevant allergen, the correlation was not good, although the

neutrophils increased in response to the nonspecific stimulus. These data indicate that there tends to be a positive correlation between mucosal scrapings and lavages for cell number and percentage.

Biopsy

The most common site of biopsy is the lower edge of the inferior turbinate. It is important to know the site of the biopsy since the mucosal lining changes from the squamous type anteriorly to the ciliated, columnar epithelium in the middle and posterior parts of the nasal cavities. Anesthesia is necessary and a vasoconstrictor agent is often required. A disadvantage of the biopsy procedure is that it is too traumatic to repeat in a serial fashion. The major advantage is that it allows examination of not only the superficial epithelium, but also the basement membrane and the submucosal components.

The leukocytes found in nasal lavage fluid have been compared to those in biopsies following antigen challenge. In allergic patients after challenge, in both the lavage fluid and the biopsy tissue, eosinophils and mononuclear cells, increased although the cell counts did not correlate. Neutrophils increased more in the lavage samples than in mucosal biopsies in both allergic and nonallergic subjects, possibly reflecting nonspecific irritation.

B. Processing Methods

Fixatives for the specimen on the slide include : air drying (not recommended); acetone; unscented hair sprays; Mota's basic lead acetate; buffered for malin; methyl alcohol; ether-95% ethyl alcohol (1:1); and 95% ethyl alcohol. The latter preparation gives excellent results.

Histological stains have variable advantages: Hansel's – eosinophils; Wright's – basophils; Wright-Giemsa-eosinophils, neutrophils, and basophils/ mast cells; Papanicolaou-epithelial cells, nuclear and cytoplasmic changes; toluidine blue-basophils/mast cells; Leishman's-eosinophils; alcian yellow-mast cells; Randolph's-eosinophils; alcian blue-basophils/mast cells; May-Grunwald-neutrophils.

C. Modes of Evaluation

Light Microscopy

Two evaluation methods of the cytogram can be used. One is a quantitative or semiquantitative assessment of the specimen, graded as a mean of cells per high-power fields, or qualitatively on a scale of 0-4+. The other method involves making a percentage calculation of specific leukocytes, e.g., 10% eosinophils. The quantitative or

semiquantitative method is generally more informative. The following part will be detailed about the nasal cytology and clinical condition.

A. Normal Subjects

The normal nasal mucosal cytology of infants, children, and adults consists of numerous epithelial cells including ciliated columnar, nonciliated columnar, goblet, and basal cells. An adequately sampled specimen will always contain some ciliated cells and goblet cells. There are usually no eosinophils or basophilic cells within the superficial layer above the basement membrane. A moderate number of neutrophils and a few bacteria can be seen, especially if the specimen is taken from the anterior portion of the inferior turbinate.

B. Allergic Rhinitis

The immunologically sensitized patient develops an immediate nasal response, and in over 50% of patients, a late-phase response with allergen provocation. Pelikan and Pelikan-Filipek studied the cytology of these responses. The cells seen in the blown secretions of 102 patients were evaluated for their immediate nasal response as defined by a period of 0-120 min after allergen challenge. The positive symptom responses were accompanied by significant changes in the count of eosinophils (increase followed by decrease) in 67%, of basophilic cells (decrease) in 13% and of neutrophils

(decrease followed by increase) in 40%. No significant changes in the count of individual cell types in nasal secretions were found during most cases of 68 negative immediate nasal responses, or during any of the 102 saline control challenges. Increase in eosinophils immediately following allergen challenge has also been described by other investigators.

The cells of nasal secretion were also examined 4-12 hr following antigen challenges in 164 allergic rhinitis patients for their late nasal response. The 104 positive late nasal responses were accompanied by significant changes in the count of eosinophils in 58% of the cases (increase immediately before and decrease during appearance of the late response), in basophils in 8% (slight increase during appearance of the late response), and in neutrophils in 84% (increase immediately before and decrease during appearance of the late response and increase again during resolution of the late response). Most of the 60 cases of negative allergen response were not accompanied by significant changes in the count of individual cell types and no changes were recorded during saline-control challenges.

Nasal lavage studies have also confirmed the changes of inflammatory cells during the late-phase reaction. The pattern of influx varied among individuals, but in general eosinophils increased within 1-2 hr after challenge and peaked (in contrast to Pelikan's work) at 7-10 hr, neutrophils increased somewhat later than eosinophils and

represented the greatest number of infiltrating cells in the late response, and basophilic cells also increased significantly but did not exceed 1% of the total cells.

Natural Allergen Exposure

The cellular response of the allergic mucosa to natural allergen exposure has also been studied. During a symptom-free period, patients with sensitization to mites and a detectable level of dust mite allergen present were evaluated and compared to healthy volunteers. All allergic patients showed a wide infiltration of inflammatory cells in the nasal mucosa, especially neutrophils, but also eosinophils and metachromatic cells. This suggests that with low-level allergen exposure, a subclinical minimal persistent inflammation is induced, even during clinical latency.

Pipkorn and co-workers studied 10 patients with isolated birch-pollen allergy from a symptom-free state before, and then in the symptomatic state during, the birch-pollen season. As the birch pollen increased, and the symptoms increased, the percentage and total number of eosinophils from the nasal lavages increased. The number of eosinophils increased 20-fold. The total number of basophilic cells found imprinted on plastic strips also significantly increased during the pollen season, but did not start to appear after until 4-5 days of pollen

exposure. A tendency to ward an increase in the number of neutrophils was noted at the peak of the pollen season.

Clinical Findings

Increased numbers of eosinophils are found in the nasal mucosa in active allergic disease. In university students, schoolchildren, and infants, a highly significant correlation has been shown with nasal secretion eosinophilia and evidence of allergy such as nose rubbing, sneezing, sniffing, runny nose, and wet and swollen turbinates. The degree of nasal eosinophilia appears to correlate with the extent of allergen exposure and with symptoms in allergic rhinitis. In particular, the increase in eosinophils generally correlates well with the symptom of nasal obstruction as measured by rhinomanometry. The presence of eosinophilia also correlates with the presence of positive allergy skin tests and, along with basophilic cells, with the serum IgE level.

Regional differences in the presence of eosinophils in the nose have been observed following antigen provocation of allergic rhinitis patients with significant increases occurring only at the site of challenge. Gristwood noted eosinophils in 100% of adult patients with perennial allergic rhinitis in specimens from both the middle and inferior turbinates. However, more cells were found in the middle

turbinate specimens. Adult allergic rhinitis patients in another study and eosinophils in 90% of the specimens obatined from the ethmoid region and muxillar sinus mucosa, 80% and 40% of specimens from the middle and inferior turbinates, respectively, and 50% of nasal secretions.

Surveying for possible variability of eosinophil detection from each nostril a clinically symptomatic patients. Kaufman et al found a concordance between nostrils for the absence or presence (>8%) of nasal secretion eosinophils in 90% and 80%, respectively, but with an overall detection rate for eosinophils >90%.

Eosinophils are found in allergic disease at all ages. In 186 children under 6 years of age referred to a group of pediatric allergists, 42% of blown secretions were positive (>10% of leukocytes). Ninety-five percent of the positive nasal smears were obtained in children determined to be atopic, 5% in an undecided group, and none in the nonatopic group.

In a prospective study of infants with a strong family history of allergic disease, it has been shown that both eosinophils and basophilic cells increase in number in nasal scrapings of allergic children between birth and 4 year of age but are uncommon in children with no allergic disease. Fifty children, age 2-7 years, were

categorized into five groups of 10 subjects as normal, allergic rhinitis, nonallergic rhinitis, allergic with otitis media, and nonallergic with otitis media by history, physical examination, allergy skin testing, and tymponometry. Adequate cytological samples were obtained from all subjects via the Rhino-Probe. Allergic groups had significantly more eosinophils than other groups. Basophilic cells were also increased in the allergic children compared to nonallergic children but the difference was significant in those with allergic rhinitis.

Miller et al. confirmed the diagnostic usefulness of the nasal smear for eosinophilia in 177 children aged 4-15 years. Significant nasal smear eosinophilia (> 4%) was observed by either nose-blowing or scraping techniques in 69% of children with seasonal allergic rhinitis (n = 65) and 12% with nonallergic rhinitis (n = 70). In their study, although the nose-blowing and scraping techniques evidenced an equal sensitivity of 70% and specificity of 94% almost 40% of children could not provide blown specimens.

In a study of adults, 19-72 years of age, Lans and co-workers (24) reported that with Rhino-Probe scrapings 43% of patients with allergic rhinitis had over 20% of sampled cells that were eosinophils. No nasal eosinophilia of this magnitude was seen in a control population or in patients with nonallergic rhinitis. Thus, in their study, the sensitivity of finding increased eosinophils in allergic rhinitis was

43%, the specificity was 100%. In another study of 210 adults with sensonal allergic rhinitis using the Rhino-Probe scraping technique, eosinophils were present in 81%, basophilic cells in 42%, neutrophils in 64%, and bacteria in 28% with at least 1 + grading. The frequency of neutrophil-positive and bacteria-positive specimens in patients with allergy is noteworthy, although this no doubt represents noninfectious inflammation; whether there is any presence of infectious inflammation requires further investigation.

Lim, Taylor, and Naclerio performed nasal lavages to study the cytology in the secretions of normal subjects, perennially allergic subjects, and seasonally allergic subjects asymptomatic and out of season and after an allergen challenge. The predominant leukocyte in all subjects was the neutrophil. Perennially allergic subjects specimens contained significantly more total cells, neutrophils, and eosinophils than specimens from the normal subjects. Seasonally allergic subjects at baseline had significantly more total cells and neutrophils than normal subject and perennially allergic subjects. After an antigen challenge, the normal subjects did not have a significant change in their cell number or pattern. In contrast, the seasonally allergic subjects showed a significant increase in the number of lavage eosinophils. Although the total number of cells and number of neutrophils increased following challenge, the change did not reach statistical significance.

The normal basophilic cell content of the nose is about 200-400 cells/mm^3 of mucosa. The great majority of these cells are located in the lamina propria. Some patients with chronic rhinitis have more than 2000 basophilic cells/mm^3 as the only histological abnormality. They have been referred to as having “nasal mastocytosis”.

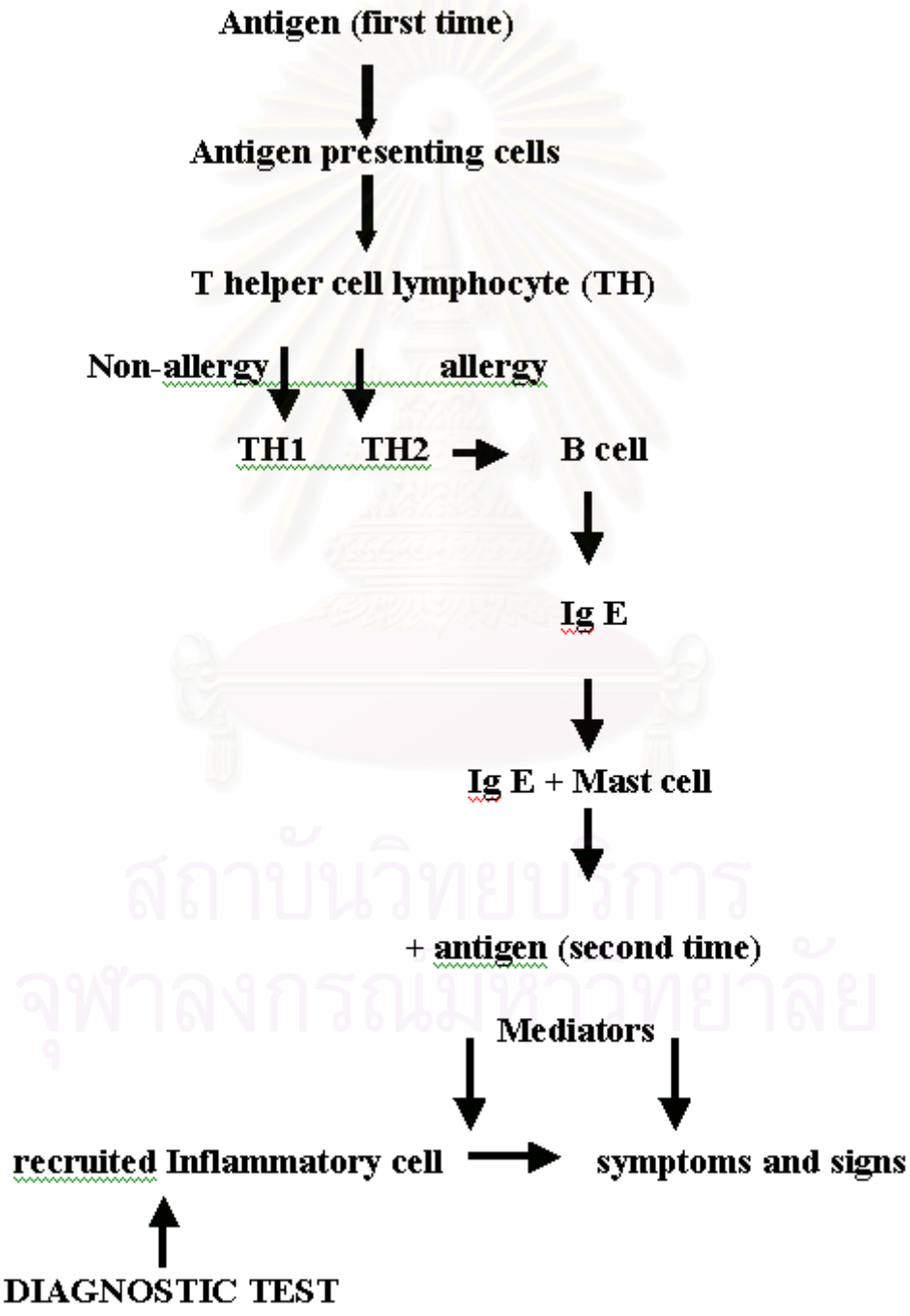
Basophilic cells have been identified as being biologically active in allergic reactions. As the number of basophilic cells increases, the nasal symptoms become more severe and provocative challenge response usually becomes stronger. The number of basophilic cells also correlates with nasal eosinophilia in allergic rhinitis. The finding of these cells and/or eosinophils increases the sensitivity of the test for confirming an allergic diagnosis to nearly 80%.

Because of various techniques for nasal cytology, different population selected and some flaws in other studied, we need to conduct this study to find out whether nasal cytology is useful and appropriate for diagnosis of allergic rhinitis in children or not.

CHAPTER 3

3. CONCEPTUAL FRAMEWORK

3.1 Figure 1



3.2 RESEARCH QUESTION

Primary Question :

Can nasal cytology be used as a diagnostic test for allergic rhinitis in pediatric patients with rhinitis for 2 or more weeks with an expected sensitivity of 80% and specificity of 90% at outpatient department (OPD) in Siriraj hospital

Secondary Question :

How high are the prevalence of allergic rhinitis and non-allergic rhinitis with eosinophilia syndrome (NARES) in Thai children with rhinitis for 2 or more weeks?

3.3 RESEARCH OBJECTIVES

1. To evaluate the accuracy, predictive values and likelihood ratio of nasal cytology test in the diagnosis of allergic rhinitis in pediatric patients with rhinitis for two or more weeks.
2. To determine the prevalence of allergic rhinitis and non-allergic rhinitis with eosinophilia syndrome in Thai children with rhinitis for 2 or more weeks.

3.4 OPERATIONAL DEFINITION

Gold standard:

The patients who were diagnosed to suffer from allergic and non-allergic rhinitis.

Allergic rhinitis:

The patients who had pasty history of the following symptoms i.e. nasal congestion, clear runny nose, sneezing, itching or clear post-nasal drip on most days for at least 4 weeks when they did not have colds, the flu or bacterial sinusitis; and had positive skin prick tests to at least one inhalant allegen.

Non-allergic rhinitis:

This group consisted of three subgroups as follows

1. *The sensitized patients* who had no history of the following symptoms i.e. nasal congestion clear runny nose, sneezing, itching or clear post nasal drip when they did not have colds, the flu or bacterial sinusitis but had positive skin prick test.

2. *Non-allergic rhinitis with eosinophilia syndrome*: The patients who had positive history of chronic nasal symptoms and positive nasal cytology test for eosinophil but negative skin prick test.

3. *Miscellaneous group* : This subgroup consisted of the patients with or without history of chronic nasal symptoms who had negative skin prick test eg. recurrent upper respiratory tract infection, adenoid hypertrophy, basophilic non-allergic rhinitis etc.

Positive skin prick test:

wheal reaction of 2 mm greater than negative control within 20 minutes.

Bacterial sinusitis:

any of the following symptoms: purulent nasal or postnasal secretions, persistent unilateral nasal stuffiness or fetid breath.

CHAPTER 4

4. RESEARCH METHODOLOGY

4.1 RESEARCH DESIGN

This study was designed to be a diagnostic test, (a descriptive study design).

4.2 POPULATION

Target population:

pediatric patients less than 12 years of age with rhinitis for 2 or more weeks in Thailand.

Study population:

pediatric patients less than 12 years of age with rhinitis for 2 or more weeks at out-patient department in Siriraj Hospital.

Inclusion criteria:

1. Children aged less than 12 years.

2. Symptoms and/or signs of rhinitis (itching, sneezing, runny nose, blocked nose, or postnasal drip) for 2 or more weeks.

3. Symptoms of recurrent rhinitis with a symptom free period of less than 7 days and the total symptom days are 2 or more weeks.

4.The agreement of the parents or guardian for the patients to participate in.

Exclusion criteria:

1. Fever within a week prior to the study day.

2. Medication that affected nasal cytology test and had not been suspended for at least one week before the study day. (oral and topical nasal steroid, and anti-leukotriene receptor antagonist.)

3.The pediatric patients who could not cooperate on physical examination

during the study.

4.The patients who were suspected of having food induced rhinitis by history or positive skin prick tests for food allergens.

5. The patients who were suspected of bacterial sinusitis.

4.3 SAMPLE SIZE

$N = Z^2_{\alpha} P(1-P)/\delta^2$

N = Number of patients with allergic rhinitis

Z_{α} = Z-value at the level of α error (0.05)

= 1.96

P = Expected sensitivity for diagnostic test.

= 0.80

δ = Error of sensitivity that can be accepted.

= 0.1

N = (1.96)(1.96)(0.8)(0.2) / (0.1)(0.1) = 62

The assumed prevalence of allergic rhinitis in this study population was about 50%.

The total number of patients enrolled was = 124 subjects.

Therefore the sample size of this study was 124 subjects and this sample size was big enough to detect specificity at 90% or more as well. The assumed sensitivity and specificity of this test were from a combined result of nasal eosinophil and basophilic metachromatic cell examination as a parallel test

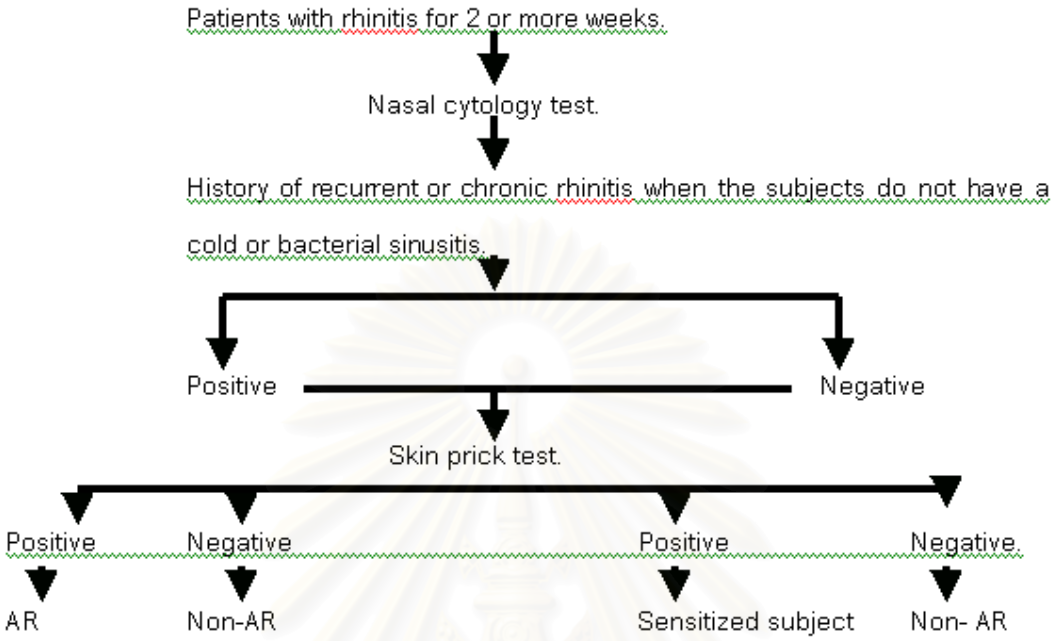
4.4 STUDY PROCEDURE

The eligible patients were enrolled and the following tests were performed :

1. Nasal cytology test by a doctor who was unaware of the diagnosis because the specimens were coded and kept in a slide box until three to five slides were reached and then examined.
2. Allergy testing by one doctor who was unaware of the results of the nasal cytology test.
3. History of chronic rhinitis was checked and recorded.

This procedure is shown in the following diagram.

Fig 2. The study procedure diagram.



METHODS

4.4.1 The details of the methods of nasal cytology tests

(a) sampling methods:

In this study, we used a plastic curette (Rhinoscrape, local products, Allertech) to sample nasal specimen of both the secretions and surface epithelium under direct visual inspection. This instrument is routinely used in clinical practice and has many advantages as mentioned in the literature review. The nasal specimen from both nasal sides could, with minimal trauma, be easily obtained by scraping the surface in the middle third of the inferior turbinate.

(b) Processing methods.

The specimen was transferred to a slide. The cupped tip was tilted onto the glass and the wet contents of the sample spreaded over a small area. The specimen was visible to the naked eye on the microscope slide to check whether it was adequate or not. Wright Giemsa solution was used for histological stains. Staining methods were as follows

1. Drip Wright-Giemsa (volu-sol) solution on the slides for 3-5 minutes.
2. Drain the excess stain then dip the slides in volu-sol buffer or distilled water for a few seconds
3. Drain the excess buffer or distilled water, and air dry specimens.
4. Scan at low power to determine whether the specimen had an adequate number of non-squamous epithelial cells and good quality stain.

(c) Evaluation

Add a drop of immersion oil to the specimen and scan at low power (x100) to determine whether the specimen had an adequate number of non-squamous epithelial cells and a good quality stain (the cytoplasmic staining of epithelial cells was light blue, the cytoplasmic granules of eosinophil stained bright red

and the cytoplasmic staining of neutrophil was dark blue). The nasal cytogram was then viewed at high power (x1000). The two cell types seen in the stained specimen were examined and graded as a mean of cells per 10 high power fields. (Appendix 1)

4.4.2. Allergy testing

2.1 Prick method

Allergy skin testing was performed by prick method using a 26 gauge needles on the upper back or forearms. Panels of common aeroallergens for allergic patients in Thailand include *Dermatophagoides pteronyssinus, Dermatophagoides farinae,* American cockroach, Johnson grass, Bermuda grass, Alternaria, Cladosporium, Cat dander, Dog hair and House dust (Center laboratories, Post Washington, NY). A positive reaction was regarded as being 2 mm or greater than the negative control. Histamine phosphate (1mg/ml) was used as positive control and 0.9% normal saline as negative control.

4.5 MEASUREMENT

Two instruments used in this study were skin prick test for allergy testing and eosinophil and basophilic matachromatic cell grading for nasal cytology test. Therefore, reliability and validity were determined and shown as follows.

1) **Reliability**

Skin prick test

Two pediatric patients with allergic rhinitis and one adult asthmatic were invited to repeat skin prick test within two weeks. Eight points for allergens, one point for positive control and one point for negative control were performed on their forearms without knowing where or which the allergens were. Skin reaction with wheal size of 2mm or greater than negative control within 20 minutes was considered positive.

The end results of this study we needed to interpret were positive or negative. Therefore, the kappa value was determined and the results are shown in table 1

Table 1 Intra-examiner reliability for skin prick

2nd test results	1st test results	
	Positive	Negative
Positive	11	0
Negative	1	18

$$\text{Kappa} = \frac{0.97 - 0.53}{1 - 0.53} = \frac{0.44}{0.47} = 0.94$$

Nasal Cytology test

The reliability of this test could be determined by intraclass correlation or kappa statics but the end results we needed to interpret were positive or negative. Therefore, the kappa value was chosen to test reliability. Twelve slides were randomly selected to be examined twice at least one month apart by a single operative whereas on other occasions two operatives were asked to perform the grading. The results of this grading of eosinophil and basophil are shown in Table 2 and also the kappa values. The results were found to be excellent as shown in table 3.

Table 2 . Grades of eosinophil and basophilic cells

(a) Single operative (Eosinophil/ Basophil)

(b)

1st	0 / 0	1+ / 2+	4+ / 0	0 / 1+	3+ / 1+	0 / 0
2nd	0 / 0	1+ / 2+	4+ / 0	0.5 / 1+	3+ / 1+	0 / 0
1st	0 / 0	2+ / 0.5	0 / 0	0 / 0	1+ / 3+	0.5+ / 0.5+
2nd	0 / 0.5	2+ / 2+	0 / 0	0.5 / 0	1+ / 2+	0.5+ / 0.5+

(b) Different operatives (Eosinophil/ Basophil)

1st	1/0.5	1/3	0.5/0	4/1	0.5/0	0 / 0
2nd	0.5/0	2/2	0 / 0	4/0	0.5/0	0 / 0
1st	0 / 0	2/1	4/0	0/0	4/1	0/0
2nd	0.5/1	2/2	2/2	0/0	2/0	0.5/0.5

Table 3 Intra and inter - observer reliability for nasal cytology test.

(a) Single operative

The cut-off point at grade 1

	1st test results	
2nd test results	**Positive**	**Negative**
Positive	6	0
Negative	0	6

Kappa = $\frac{1-0.51}{1-0.51} = \frac{0.49}{0.49} = 1$

The cut-off point at grade 2.

	1st test results	
2nd test results	**Positive**	**Negative**
Positive	5	0
Negative	0	7

Kappa = $\frac{1-0.51}{1-0.51} = \frac{0.49}{0.49} = 1$

(b) different operatives

The cut-off point at grade 1.

	1st test results	
2nd test results	**Positive**	**Negative**
Positive	5	0
Negative	1	6

Kappa = $\frac{0.92 - 0.5}{1 - 0.5} = \frac{0.42}{0.5} = 0.84$

The cut-off point at grade 2.

	1st test results	
2nd test results	**Positive**	**Negative**
Positive	5	0
Negative	0	7

Kappa = $\frac{1 - 0.51}{1 - 0.51} = \frac{0.49}{0.49} = 1$

2) Validity.

Nasal cytology test:

The validity was determined and shown after the study had been completed.

Skin prick test:

This test is recommeded as the primary test for the diagnosis of IgE-mediated alllergic diseases and for research purpose by the European Academy of Allergology and clinical Immunology and the U.S. Joint council of Allergy, Asthma and Immunology.

4.6 DATA COLLECTION

The eligible pediatric patients, who came to OPD at Siriraj Hospital, were enrolled between June 2000 and March 2001. These patients were not randomly selected because there were not many patients who were compatible with inclusion criteria and we assumed that the patients came to hospital at random. The following detail were recorded.

Administrative variables

- name
- address
- identification number

Zero state variables

- age

- sex
- duration of the symptoms of rhinitis on this episode
- past history of nasal symptoms and duration before this episode

Outcome variables

- total number in each cell type.
- grades of nasal cytology for each kind of cells ; Eosinophils, Basophilic metachromatic cells.
- the patients who were diagnosed of having or not having allergic rhinitis.
- the patients who were diagnosed as non-allergic rhinitis with eosinophilia syndrome.

4.7 DATA ANALYSIS AND STATISTICAL TEST

Analysis of outcome variables.

1. The sensitivity, specificity, predictive value and accuracy:

These values were evaluated after the best cut-off point of nasal cytology tests for eosinophil and basophilic metachromatic cells had been determined. Where the highest value of the sum of the sensitivity and the specificity which was at least 90% among each level of cell grading made the best cut-off point for each test. The sensitivity, specificity and the predictive values at that cut-off point were reported. These results were combined and categorized in two-by-two table as

a **parellel test.** These values of sensitivity, specificity, predictive values were calculated from. The combined cell finding.

2. Likelihood ratio:

Likelihood ratio was calculated and shown at each level of nasal cytology grading for each kind of cells and for each level of combined results.

3. Relative frequency:

The relative frequency of allergic rhinitis and NARES were determined and shown for the prevalence.

CHAPTER 5

RESULTS

The base-line characteristics of the subjects are shown in Table 4. The median age of the subjects was 6 years 3 months and ranged from 2 years 5 months to 11 years 3 months. The age and sex between allergic rhinitis group and non-allergic rhinitis group were comparable as shown in table 4. They consisted of 83 boys and 41 girls. Of the 124 subjects, 70 were diagnosed to suffer from allergic rhinitis.

Table 4 Base-line Characteristics of the 124 subjects

Characteristic	All subjects (n = 124)	AR (n = 70)	Non – AR (n = 54)
Age (month)			
mean (± SD)	76.12 (27.04)	80.63 (25.48)	70.28 (28.12)
median (range)	75 (29 – 135)	78 (42 – 135)	69.5 (29 – 121)
Male (%)	67%	69%	65%
Duration of rhinitis on the visiting day			
0.5 – 1 mo	79 (63.71 %)	37 (52.86 %)	42 (77.78 %)
1.1 – 6 mo	35 (28.23 %)	23 (34.29 %)	11 (20.37 %)
> 6 mo	10 (08.06 %)	9 (12.85 %)	1 (01.85 %)
Number of allergen sensitization			
One allergen		57.14 %	3.71 %
Two allergens		24.29 %	1.86 %
> two allergens		18.57 %	
skin prick test results			
Dust mite		91.43 %	5.56 %
Cockroach		28.57 %	1.86 %
Cat dander		12.86 %	
Bermuda grass		12.86 %	
Johnson grass		10 %	
Dog hair		2.85 %	
House dust		1.42 %	

AR = allergic rhinitis
Non - AR = non allergic rhinitis

The number of the subjects in each grade of eosinophil and basophil are shown in table 5 and 6. None of the subjects in non-allergic rhinitis group were in grade 3 and 4 whereas it was comparable between both groups in grade 0.5 and 1.

Table 5 The number of the subjects in each grade of eosinophil

Grade of eosinophil	AR(%)	Non-AR(%)
4	11 (15.71)	0
3	5 (7.14)	0
2	17 (24.29)	2 (3.70)
1	12 (17.14)	7 (12.97)
0.5	16 (22.86)	16 (29.63)
0	9 (12.86)	29 (53.70)
Total	70 (100)	54 (100)

Table 6 The number of the subjects in each grade of basophilic cells

Grade of basophil	AR(%)	Non-AR(%)
4	4 (5.71)	0
3	16 (22.86)	0
2	18 (25.71)	4 (7.40)
1	14 (20.00)	8 (14.82)
0.5	8 (11.43)	8 (14.82)
0	10 (14.29)	34 (62.96)
Total	70 (100)	54 (100)

The sensitivity, specificity, predictive values and likelihood ratio of eosinophil and basophil at each level are shown in table 7 and 8 .

Table 7 The sensitivity, specificity, predictive values and likelihood ratio in each grade of eosinophil

Cut - off Point at grade	Cumulative number of subjects including preceding grade		Sensitivity	Specificity	PPV	NPV	LR
	AR	Non AR					
4	11	0	15.71	100	100	47.79	-
3	16	0	22.86	100	100	50.00	-
2	33	2	47.14	96.33	94.29	58.43	6.56
1	45	9	64.29	64.29	83.33	64.29	1.32
0.5	61	25	87.14	53.70	67.78	76.32	0.72
0	70	54	100	0	56.45	0	0.24

PPV = Positive predictive value

NPV = Negative predictive value

LR = Likelihood ratio

Table 8 The sensitivity, specificity, predictive values and likelihood ratio in each grade of basophilic cells

Cut-off Point at grade	Cumulative number of subjects including preceding grade		Sensitivity	Specificity	PPV	NPV	LR
	AR	Non AR					
4	4	0	5.71	100	100	45	-
3	20	0	28.57	100	100	51.92	-
2	38	4	54.29	92.59	90.48	60.98	3.47
1	52	12	74.29	70.37	81.25	70.00	1.33
0.5	60	20	85.71	33.33	75.00	77.27	0.77
0	70	54	100	0	56.45	0	0.23

The objective of this study is used for confirmation. Therefore, specificity is important and should be higher than 90%. Nasal cytology test for eosinophil and basophil should be set at grade 2 for both types of cells. The positive and negative predictive values are shown in table 7 and 8

Both nasal eosinophil and basophil examination were combined as a parallel test by using the cut-off point at grade 2 for each cell type. The sensitivity and specificity were determined as shown in table 9 and 10. The sensitivity was much higher whereas the specificity was slightly lower in the combined results than those in a single one. The likelihood ratio for each level of the combined results that were

determined are shown in table 11. The area under curve (ROC) of combined results was 0.918 whereas the area under curve of eosinophil and basophil cell examination were 0.812 and 0.829 respectively.

Table 9 The number of subjects in different groups showing the nasal cytology results from both AR and Non AR

(a)

Basophil examination	Eosinophil examination	
	Positive	Negative
Positive	15	18
Negative	23	14

Allergic rhinitis group (AR)

(b)

Basophil examination	Eosinophil examination	
	Positive	Negative
Positive	1	1
Negative	3	49

Non-allergic rhinitis group (Non-AR)

Table 10 The results of the combination of eosinophil and basophilic cells examination.

Test results	AR	non AR
Eosinophil and /or Basophil positive	56	5
Eosinophil and basophil negative	14	49

Sensitivity = 80% (95% CI ; 70.63-89.37)

Specificity = 90.74% (95% CI; 83.01-98.47)

Positive predictive value = 91.80% (95% CI; 84.92-98.68)

Negative predictive value = 77.78% (95% CI; 67.51 – 88.05)

The prevalence of allergic rhinitis = 56.45% (95% CI; 47.72 – 65.18)

Table 11. The likelihood ratio for each level of combined results.

Levels of combined results	AR	Non- AR	Likelihood ratio
Both Eo and Ba negative	14	49	0.22
Either Eo or Ba positive	41	4	7.91
Both Eo and Ba positive	15	1	11.57

Some subjects had positive nasal cytology test in both eosinophil and basophil but the level was lower than grade 2. Therefore this criterion was added to the former combined results as a parallel test again, the sensitivity and specificity were determined by using grade 1 for both cell types. The results are shown in table 12.

Table 12 The results of the three combined criteria

Test results	ARNon	AR
Eo grade2 or Ba grade 2 or both Eo grade 1 and Ba grade 1: POSITIVE	58	8
Eo and Ba grade 2 and both Eo and Ba grade 1: NEGATIVE	12	46

CHAPTER 6

DISCUSSION

In practice, when we perform this test, two kinds of cells may be seen in the same subject in equal numbers and sometimes one cell type is easily seen over the other. This study shows that the nasal cytology test for eosinophil or basophilic cells gives good specificity but low sensitivity (table 7 and 8). When both results were combined as a parallel test, the sensitivity was much higher and the specificity was slightly lower but still high (table 10). Another criterion for positive test was tried to combine this test as a parallel test again, but the results were not good enough (table 12). Therefore, a combination of these two cell types as a parallel test should be performed on the patients.

When comparing these results with other studies which I have mentioned in the literature review, some have lower sensitivity or specificity and others have higher results. It depends on the selected subjects and the techniques used in those studies. One study, that was carried out on pediatric patients, utilized Rhinoprobes for specimen sampling and Wright Giemsa solution for staining the specimen as our study. Jirapongsananuruk et al determined the diagnostic value of the nasal cytology test and demonstrated that only eosinophil or basophil examination had

higher specificity compared with sensitivity and also better positive predictive value. The sensitivity of 91.67 %, specificity of 100%, positive predictive value 100% and negative predictive valve of 91.1% that were reported in her study were higher than our results.

The subjects with allergic rhinitis in that study, who were recruited from the allergy clinic (tertiary care unit) might have had more severe symptoms than our subjects who were recruited from OPD. The control group she studied were healthy children who did not have any symptoms as compared with our subjects who had rhinitis for at least 2 weeks similar to the index group. The criteria for diagnosis of allergic rhinitis between these two studies were similar.

The diagnosis of allergic rhinitis used in this study depended on history and a positive skin prick test to at least one inhalant allergen. Although, provocation tests are the gold standard method , they are less well standardized than skin tests and in children, who have perennial symptoms, may have nasal hyper-reactivity to any allergens or placebo. Therefore, we included all children who had classical history of sneezing, itching, rhinorrhea or stuffy nose in the morning or evening and recurrent rhinorrhea through the day. This effect would make the sensitivity lower than it should be because the number of subjects was over diagnosed as allergic rhinitis. For specificity, the result might have been lower than it should be, perhaps, because the history taken from the parents was less positive. Renzoni et al have shown that the history taken from

parents was less positive than those from children. However in this study we had 3 subjects who were diagnosed as sensitized patients, none of them was positive for nasal cytology. Another reason was that some subjects who had positive results for the nasal cytology test but negative results for the skin prick test were included in non- allergic rhinitis group. Some of these subjects (5 cases in grade 2 or more) may have been misdiagnosed, because they may have had local specific IgE production in their noses that would make them have nasal symptoms and a positive result for the nasal cytology test without reactivity to the skin prick test.

Some subjects recruited in this study had preceding histories of virus-induced rhinitis. The cellular infiltrate in the nasal mucosa during rhinovirus infection has so far received limited study. The number of polymorphonuclear leukocytes increased in the mucosa of the inferior turbinate very early (day 2) during naturally acquired colds compared with the recovery phase (day 14) in the same individuals. An increased number of polymorphonuclear leukocytes was also noted in volunteers with rhinovirus induced colds on the first and second days when compared to sham-inoculated controls. Fraenkel et al did not find any change in the number of polymorphonuclear leukocytes by immunohistochemistry on day 4 of illness in volunteers with rhinovirus induced colds compared to normal controls. Infiltration of polymorphonuclear leukocytes into (and through) the nasal mucosa may be a very early and transient

event. Within 24 hours of rhinovirus inoculation, Naclerio et al found an increase in polymorphonuclear leukocytes in nasal washes obtained every 4 hours around the clock the number had already begun to decrease by day 3.

Recently, Avial et al have shown that the patients with allergic rhinitis received nasal challenge with allergen or placebo over the week before nasal inoculation with rhinovirus type 16. Increase in neutrophils and interleukin 6 and 8 concentrations in nasal lavage fluid were similar in both groups until day 15 when compared with eosinophil, in which an increase was not observed. Therefore, the time we perform nasal cytology test should be longer than 2 weeks after history of virus induced rhinitis. This may affect our results and may lower the sensitivity of the test (The history was suggestive to viral infection in 4 cases).

Some subjects, who were diagnosed as non-allergic rhinitis, had eosinophil or basophil in their nasal mucosa from grade 0.5 to 2. These patients may develop allergic disease in the future. In a prospective study of infants with a strong family history of allergic disease carried out by Zeiger and Heller, it was reported that both eosinophils and basophilic cells increases in number in nasal scrapings of allergic children from age 4 months through 4 years but were uncommon in children with no allergic disease. Although, the limitations of the gold standard method used in this study and the excellent results of intra-observer and inter-observer reliability,

this study shows that the nasal cytology test has good sensitivity and specificity and can be generalized to the pediatric patients who have nasal symptoms for longer than 2 weeks even to a mild degree. And the pediatric patients who have a positive result to nasal cytology test will have about 84% the chance of being sensitized to dust mite (positive predictive value x percentage of allergic rhinitis patients with sensitization to dust mite). It should also be suggested to these patients that they avoid dust mite with the standard method. Although, the positive predictive value was high (91.80%), this means that about 9 % percent of the patients who were diagnosed as allergic rhinitis by nasal cytology test will be treated incorrectly the treatment with nasal corticosteroid looks harmful for the patients who are not allergic rhinitis , but it is helpful in these cases because nasal corticosteroid will decrease nasal eosinophil and basophil and also nasal symptoms .

CHAPTER 7

CONCLUSION

Several studies have reported the correlation between nasal eosinophilia and symptoms in allergic rhinitis. Various techniques have been utilized to confirm this results. In this study, nasal scraping and Wright-Giemsa stain were chosen to perform on the pediatric patients aged between 29 months and 135 months who had symptoms of rhinitis for 2 or more weeks. The nasal cytology test used was the combination between nasal eosinophil and nasal basophilic cells. The results of this study were as follows the sensitivity of 80%, specificity of 90.70%, positive predictive value of 91.80% and negative predictive value of 77.78%. The prevalences of allergic rhinitis, non-allergic rhinitis with eosinophilia syndrome and basophilic non-allergic rhinitis in the pediatric patients with rhinitis for 2 or more weeks were 56.45%, 1.6% and 2.4% respectively. Further study is required to confirm these values because the sample size in this study was too small. Because of the good results and the

excellent reliability of the test, this test is useful and appropriate to confirm the diagnosis of allergic rhinitis.

REFERENCES:

1. Juniper EF, Guyatt GH, Dolovich J. Assesment of quality of life in adolescents with allergic rhinoconjunctivitis: development and testing of a questionnaire for clinical trials. J Allergy Clin Immunol 1994;93:413-23.
2. Juniper EF, Hawland WC, Roberts NB, Thompson AK, King DR. Measuring quality of life in children with rhinoconjunctivitis. J Allergy Clin Immunol 1998;101(2 pt 1):163-70.
3. Turner RB. Epidemiology, pathogenesis and treatment of the common cold. Ann Allergy Asthma Immunol 1997; 78: 531-9.
4. Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, et al. Community study of role of virus infection in exacerbations of asthma in 9-11 year old children. Br. Med. J. 1994; 310:1225-8.
5. Frisell E: A comparison between the sedimentation rates of children in day-nurseries and children cared for in their homes. Acta Paediatr 1948;35:1-9.
6. Hesselvik L: Respiratory infections among children in day nurseries. Acta Paediatr Scand 1949;74(suppl):96-103.
7. Loda FA, Glezen WP, Clyde WA: Respiratory disease in group day care. Pediatrics 1972;49:428-37.

8. Strangert K: Respiratory illness in preschool children with different forms of day care. Pediatrics 1976;57:1191-6.
9. Doyle AB: Incidence of illness in early group and family day-care. Pediatrics 1976;58:607-13.
10. Wald ER, Guerra N, Byers C. Upper respiratory tract infections in young children: duration and frequency of complications. Pediatrics 1991;98:129-37.
11. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin test reactivity to allergens. N Engl J Med 1989;320:271-7.
12. Howarth PH. The cellular basis for allergic rhinitis. Allergy 1995;50(Suppl 23):6-10.
13. Fokkens WJ, Vinke JG, Kleinjan A. Local IgE production in nasal onucosa : A review. Am J Rhinol 2000; 14: 299-303.
14. Bradding P, Feather IH, Wilson S, Bardin P, Holgate ST, Howarth PH. Immunolocalisation of cytokines in the nasal mucosa of normal and perennial rhinitis subjects: the mast cell as a source of IL-4, IL-5 and IL-6 in human allergic inflammation. J Immunol 1993; 151: 3853-65.
15. Bradding P, Feather IH, Wilson S, Holgate ST, Howarth PH. The effects of seasonal exposure to allergic nasal cytokine

immunoreactivity and its modulation by fluticasone propionate. Thorax 1993; 48:1059.

16. Howarth PH, Holgate ST. Basic aspects of allergic reactions. In: Naspitz C, Tinkelman DG. Eds. Childhood Rhinitis and Sinusitis: Pathophysiology and Treatment. New York: Marcel Dekker, 1990; 1-35.

17. Vaheri E. Nasal allergy with special reference to eosinophilia and histopathology. Acta Allergol 1956; 10:203-11.

18. Mullarkey MF, Hill JS, Webb DR. Allergic and non-allergic rhinitis: their characterisation with attention to the meaning of nasal eosinophilia. J Allergy Clin Immunol 1980; 65:122-26.

19. Otsuka H, Denburg JA, Dolovitvh J, Hitch D, Lapp D, Rajan RS, et al. Heterogeneity of metachromatic cells in human nose: significance of mucosal mast cells. J Allergy Clin Immunol 1985;76:695.

20. Okuda M, Otsuka H, Kawabori S. Studies on nasal surface basophilic cells. Ann Allergy 1985; 54:69.

21. Bascom R, Wachs M, Naclerio RM, Pipkorn U, Galli SJ, Lichtenstein LM. Basophil influx occurs after nasal allergen challenge: effects of topical corticosteroid pretreatment. J Allergy Clini Immunol 1988; 81: 580-9.

22. Kirschenbaum AS, Kessler SW, Gott JP, Metcalfe DD. Demonstration of the origin of human mast cells from CD34+ bone marrow progenitor cells. J Immunol 1991;146:1410-5.

23. Charance M, Herbeth B, Kauffman F. Seasonal patterns of circulating basophils. Int Arch Allergy Appl Immunol 1988;86:462-4.

24. Eriksson NE. Diagnosis of reaginic allergy with house dust, animal dander and pollen allergens in adult patients. Int Archs Allergy appl Immun 1977; 53: 441-9.

25. Wright AL, Holberg CJ, Martinez FD, Halonen M, Morgan W, Taussig LM. Epidemiology of physician-diagnosed allergic rhinitis in childhood. Pediatrics 1994; 94: 895-901.

26. Wolthers OD, Pedersen S. short term growth in children with allergic rhinitis treated with oral antihistamines and intranasal glucocorticoids. Acta Pediatr 1993;82:635-40.

27. Meltzer EO, Schatz M, Zeiger RS. Allergic and non-allergic rhinitis. In: Middleton E Jr, Reed CE, Ellis EF, eds. Allergy: Principles and Practice, 3rd ed. St Louis: CV Mosby, 1988; 1253-89.

28. Hansel FK. Observation on the cytology of the secretions in allergy of the nose and paranasal sinuses. J Allergy 1934;5:357-66.

29. Hastie R, Heroy JH, Levy DA. Basophil leukocytes and mast cells in human nasal secretions and scrapings studied by light microscopy. Lab Invest 1979;49:541-54.

30. Bryan WTK, Bryan MT. Cytologic diagnosis in otolaryngology. Trans Am Acad Ophthalmol Otolaryngol 1959;63:597-611.

31. Pipkorn U, Karlsson C. Methods for obtaining specimens from the nasal mucosa for morphological and biochemical analysis. Eur Respir J 1988;1:856-862.

32. Pipkorn U, Karlsson G, Enerback L. A brush method to harvest cells from the nasal mucosa for microscopic and biochemical analysis. J Immunol Meth 1988;112:37-42.

33. Jalowayski AA, Zeiger RS. Examination of nasal or conjunctival epithelial specimen. In: Lawlor GJ Jr, Fischer TJ, eds. Manual of Allergy and Immunology, 2nd ed. Boston: Little, Brown, 1988;432-34.

34. Meltzer EO, Jalowayski AA. Nasal cytology in clinical practice. Am J Rhinol 1988;2:47-54.

35. Jalowayski AA. Meltzer EO, Orgel HA. Welch MJ, Ostrom NK, Kemp JP. Nasal histamine levels in young children with allergic and non-allergic rhinitis and chronic otitis. J Allergy Clin Immunol 1991;87:146.

36. Welch MJ, Meltzer EO, Kemp JP, Orgel HA, Ostrom NK, Jalowayski AA. Comparison of two different techniques for obtaining specimens for nasal cytology. Nose-blowing vs. nasal mucosal scraping. J Allergy Clin Immunol 1991;87:144.

37. Galindo C, Jalowayski A, Meltzer E. Correlation between nasal cytogram and blown technique for the diagnosis of allergic rhinitis. Ann Allergy 1991;66:86.

38. Angel-Solano G, Shturman R. Comparative cytology of nasal secretions and nasal mucosa in allergic rhinitis. Ann Allergy 1986;56:521.

39. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF, Jr, Meyess DA, Norman PS et al. Mediator release after nasal airway challenge with allergen. Am Rev Respir Dis 1983;128:597-602.

40. Piancentini GL, Kaulbach HC, Scott T, White MV, Kaliner MA. Correlation between inflammatory cell responses in nasal mucosal scrapings and lavages after allergen challenge. J Allergy Clin Immunol 1991;87:145.

41. Brown M, Lim M, Furin M, Taylor M, Naclerio R. A comparison between antigen-induced leukocyte populations in the nasal mucosa and nasal secretions. J Allergy Clin Immunol 1991;87:145.

42. Cohen GA, MacPherson GA, Golembesky HE, Jalowayski AA, O'Connor RD. Normal nasal cytology in infancy. Ann Allergy 1985;54:112-24.

43. Bryan MP, Bryan WTK. Cytologic diagnosis in allergic disorders. Otolaryngol Clin North Am 1974;7:637-66.

44. Bickmore JT. Nasal cytology in allergy and infection. Otorhinolaryngology Allergy 1978;40:39-46.

45. Ohtsuka H, Okuda M. Important factors in the nasal manifestations of allergy. Arch Otorhinolaryngol 1981;233:227-35.

46. Miller RE, Paradise JL, Friday GA, Fireman P, Voith D. The nasal smear for eosinophils. Am J Dis Child 1982; 136:1009-11.

47. Pelikan Z, Pelikan-Filipek M. Cytologic changes in the nasal secretions during the immediate nasal response. J Allergy Clin Immunol 1988;82:1103-12.

48. Pipkorn U, Karlsson G, Enerback L. Nasal mucosal response to repeated challenges with pollen allergen. Am Rev Respir Dis 1989;140:729-36.

49. Pelikan Z, Pelikan-Filipek M. Cytologic changes in the nasal secretions during the late nasal response. J Allergy Clin Immunol 1989;83:1068-79.

50. Togias A, Naclerio RM, Proud D, Pipkorn U, Bascom R, Iliopoulos O. Studies on the allergic and non-allergic inflammation. J Allergy Clin Immunol 1988;81:782-90.

51. Ciprandi G, Buscaglia S, Pesce G, Pronzato C, Ricca V, Parmiani S et al. Minimal persistent inflammation is present at mucosal leve in patients with asymptomatic rhinitis and mite allergy. J Allergy Clin Immuno 1995;96:971-9.

52. Pipkorn U, Karlsson G, Enerback L. The cellular response of the human allergic mucosa to natural allergen exposure. J Allergy Clin Immunol 1988;82:1046-54.

53. Hansel FK. Cytologic diagnosis in respiratory allergy and infection. Ann Allergy 1966;24:564-9.

54. Wang D, Clement P, DeWaele M, Derde M-P. Study of nasal cytology in atopic patients after nasal allergen challenge. Rhinology 1995;33:78-81.

55. Orgel HA, Kemp JP, Meltzer EO, Hamburger RN. Atopy and IgE in a pediatric allergy practice. Ann Allergy 1977;39:161-8.

56. Salas A, Wilson N, Humburger RN. Relation of serum IgE level to the cell observed in the nasal cytograms. Ann Allergy 1988;60:175.

57. Lim MC, Taylor RM, Naclerio RM. The histology of allergic rhinitis and its comparison to cellular changes in nasal lavage. Am J Respir Crit Care Med 1995;151:136-44.

58. Gristwood RE. Observations on the histopathology of allergic rhinitis: regional differences in mucosal eosinophilia. J Laryngol Otol 1982;49:270.

59. Kaufman HS, Rosen I, Shaposhnikov N, Magdalen W. Nasal eosinophilia. Ann Allergy 1982;49:270-1.

60. Zeiger S, Heller S. Development of nasal basophilic cells and nasal eosinophilia from ages 4 months through 4 years in children of atopic parents. J Allergy Clin Immunol 1993;91:723-34.

61. Meltzer EO, Orgel HA, Jalowayski AA. Histamine levels and nasal cytological children with chronic otitis media and rhinitis. Ann Allergy Asthma Immunol 1995;74:406-10.

62. Connell JT. Nasal disease: mechanisms and classification. Ann Allergy 1983;50:227-35.

63. Lang DM, Howland WC, Stevenson DD. Sensitivity and features of nasal cytology in diagnosis of allergic (IgE mediated) rhinitis. Ann Allergy 1988;60:176.

64. Jirapongsananurak O, Vichyanond P. Nasal cytology in the diagnosis of allergic rhinitis in children. Annals of Allergy Asthma Immunology 1998;80:165-70.

65. Renzoni E, Forastiere F, Biggeri A, Viegi G, Bisanti L, Chellini E, et al. Difference in parental and self-report of asthma, rhinitis and eczema among Italian adolescents. Eur Respir J 1999;14:597-604.

66. Winther B, Brofeldt S, Christensen B, Mygind N. Light and scanning electron microscopy of nasal biopsy material from patients with naturally acquired common colds. Acta Otolaryngol (Stockh) 1984;97:309-18.

67. Winther B, Farr B. Turner RB, Hendley JO, Gwaltney JM, Jr, Mygind N. Histopathologic examination and enumeration of polymorphonuclear leukocytes in the nasal mucosa during experimental rhinovirus colds. Acta Otolaryngol Suppl 1984;413: 19-24.

68. Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST. Immunohistochemical analysis of nasal biopsies during rhinovirus experimental colds. Am J Respir Crit Care Med 1994;150:1130-6.

69. Naclerio RM, Proud D, Lichtenstein LM, Kagey-Sobotka A, Hendley JO, Sorrentino J, et al. Kinins are generated during experimental rhinovirus colds. J Infect Dis 1988;157:133-42.

70. Avila PC, Abisheganaden JA, Wong H, Liu J, Yagi S, Schnurr D, et al. Effect of allergic inflammation of the nasal mucosa on the

severity of rhinovirus 16 cold. J Allergy Clin Immunol 2000;105: 923-32.

Appendix 1

Grade of nasal cytology

Quantitative analysis	**Grade**
Eosinophil	
0	0
0.1 – 1.0	0.5
1.1 – 5.0	1
5.1 – 15	2
15.1 – 20.0	3
> 20	4
Basophilic cells	
0	0
0.1 – 0.3	0.5
0.4 – 1	1
1.1 – 3.0	2
3.1 – 6	3
> 6	4

Appendix 2

CASE RECORD FORM

NO..... **Date**.....

Name .... **Sex**..... **Age** ..... **HN** ....

Symptoms **Duration**

- ❒ **sneeze** .....
- ❒ **itch** .....
- ❒ **blocked** .....
- ❒ **rhinorrhea** .....
- ❒ **fever** .....
- ❒ **post nasal drip** .....
- ❒ **cough** .....
- ❒ **sore-throat** .....

Signs	**nasal turbinate**		**right**	**left**
	Inferior	congestion	❒	❒
		Inflammation	❒	❒
		pale mucosa	❒	❒
	Middle	congestion	❒	❒
		Inflammation	❒	❒
		pale mucosa	❒	❒
	Middle meatus	discharge	❒	❒
		Intact	❒	❒
Discharge	**Nasal canal**			
		watery	❒	❒
		mucoid	❒	❒
		purulent	❒	❒
	Middle meatus			
		watery	❒	❒
		mucoid	❒	❒
		purulent	❒	❒

Skin prick test ❒ **positive** ❒ **negative**

Allergen..... **grade**.....

Nasal cytology

- ❒ **Eosinophil** **cell/field** **grade**.....
- ❒ **Basophil** **cell/field** **grade**.....
- ❒ **Neutrophil** **cell/field** **grade**.....
- ❒ **Lymphocyte** **cell/field** **grade**.....
- ❒ **Goblet cell** **cell/field** **grade**.....
- ❒ **Negative**

Table 1

Quantitative analysis	**Grade**
Eosinophil	
0	0
0.1 – 1.0	0.5
1.1 – 5.0	1
5.1 – 15	2
15.1 – 20.0	3
> 20	4
Basophilic cells	
0	0
0.1 – 0.3	0.5
0.4 – 1	1
1.1 – 3.0	2
3.1 – 6	3
> 6	4

Appendix 3

แบบยินยอมเข้าร่วมการศึกษา

วันที่.....เดือน..... พ.ศ. .....

ข้าพเจ้า..... อายุ..... ปี เกี่ยวข้องเป็น ❒ บิดา ❒ มารดา ❒ ผู้ปกครอง ❒ ดช. ❒ ดญ..... อายุ..... ปี อาศัยอยู่บ้านเลขที่..... ถนน..... แขวง..... เขต..... จังหวัด.....

ได้รับทราบรายละเอียดของโครงการวิจัยเรื่อง "ประโยชน์ของการทดสอบเซลล์เยื่อจมูกในการวินิจฉัยผู้ป่วยโรคจมูกอักเสบจากภูมิแพ้ในเด็ก" โดยมีวัตถุประสงค์เพื่อศึกษาการทดสอบเซลล์เยื่อจมูก เพื่อนำมาช่วยในการวินิจฉัยผู้ป่วยสงสัยโรคจมูกอักเสบจากภูมิแพ้ โดยข้าพเจ้าจะได้รับการตรวจเยื่อจมูก 1 ครั้ง, การทดสอบภูมิแพ้ทางผิวหนัง 1 ครั้ง ซึ่งผลข้างเคียงที่อาจเกิดจากการทดสอบภูมิแพ้ทางผิวหนัง คือ ลมพิษ, เป็นลม ถ้ารุนแรงอาจบวมบริเวณใบหน้า หายใจขัด หรือช็อก ซึ่งผลข้างเคียงทั้งหมดที่กล่าวมาพบได้น้อยมาก จากการศึกษาในประชากร 16,204 ราย พบว่าไม่มีผลข้างเคียงดังกล่าวเลย

หากข้าพเจ้ามีข้อสงสัยประการใด หรือเมื่อเกิดผลข้างเคียงจากการวินิจฉัยขึ้น ข้าพเจ้าจะติดต่อกับ นพ. รณชัย วิริยะทวีกุล หมายเลขใบประกอบวิชาชีพเวชกรรม 11219 หมายเลขโทรศัพท์ที่ติดต่อ 4197052 หรือ (01) 6580596

หากผู้วิจัยมีข้อมูลเพิ่มเติมทั้งด้านประโยชน์และโทษที่เกี่ยวข้องกับการวิจัยนี้ ผู้วิจัยจะแจ้งให้ข้าพเจ้าทราบอย่างรวดเร็วโดยไม่ปิดบัง

ข้าพเจ้ามีสิทธิ์ที่จะของดการเข้าร่วมโครงการวิจัยโดยมิต้องแจ้งให้ทราบล่วงหน้าโดยการงดการเข้าร่วมโครงการวิจัยนี้จะไม่มีผลกระทบต่อการได้รับบริการหรือการรักษาที่ข้าพเจ้าจะได้รับแต่ประการใด

ข้าพเจ้าได้รับทราบจากผู้วิจัยว่า จะไม่เปิดเผยข้อมูลหรือผลการวิจัยของข้าพเจ้าเป็นรายบุคคลต่อสาธารณชน

ข้าพเจ้าได้รับทราบและได้ซักถามผู้วิจัยจนหมดข้อสงสัยโดยตลอดแล้วและยินดีเข้าร่วมในการวิจัย จึงได้ลงลายมือชื่อไว้เป็นหลักฐานต่อหน้าพยาน

ลงชื่อ.....ผู้ยินยอมหรือผู้แทนโดยชอบธรรม
(.....)

ลงชื่อ..... หัวหน้าโครงการวิจัย
(.....)

ลงชื่อ..... พยาน
(.....)

ลงชื่อ..... พยาน
(.....)

Curriculum Vitae
Ronnachai Viriyataveekul, M.D.

PERSONAL DATA

Name	: Ronnachai Viriyataveekul, M.D.
Date of birth	: May 6, 1959
Place of birth	: Bangkok, Thailand
Citizenship	: Thai
Marital Status	: Married
Office Address	: Dept of Clinical Pathology, Siriraj Hospital, Bangkok Thailand Tel. 4197052 Fax : 4197052
Home Address	: 93/1 Soi Areesumphan 2, Payathai, Bangkok, Thailand Tel. 279-5819

EDUCATION

Medical School (1993)	: Faculty of Medicine, Siriraj Hospital Mahidol University,Bangkok, Thailand
Degree	: Doctor of Medicine 1983

PROFESSIONAL EXPERIENCE

Fellowship 1992	: Fellowship in Allergy and Clinical Immunology Department of Pediatrics, Mahidol University Siriraj Hospital, Bangkok 10700, Thailand
Pediatric Residency 1986-1989	: Department of Pediatrics, Faculty, of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
BOARD CERTIFICATION	: Thai Board of Pediatrics 1/7.1989
MEDICAL LICENSURE	: Thailand No. 11219 April, 1984
SOCIETIES	: The Pediatric Society of Thailand : The Thai Medical Council

HOSPITAL AFFILIATIONS

1989-1997	: Central Hospital, Bangkok, Thailand
1998-present	: Siriraj Hospital, Bangkok, Thailand

PUBLICATION AND ABSTRACT

: R. Viriyataveekul, P. Vichyanond. Comparision of four types of needles used in allergy skin prick testing Siriraj Hosp Gaz 1995;47 suppl, 110-4.

: R. Viriyataveekul, S. Nilganuwong, S. Chaihirunkarn, et al. Comparison of Rheumatoid Factor Testing Methods: Nephelometric Assay Versus Latex Agglutination Assay. Siriraj Hosp Gaz 2000;52(2): 99-104.

: R. Viriyataveekul, P. Vichyanond, K.Pattanapanyasat et al.Peripheral Blood Type 1 and Type 2 T cells in Thai Children with Atopic Diseases. Siriraj Hosp Gaz 2000; 52(11): 742-7.