

Research letters

Hormone status of in-situ cancer in *BRCA1* and *BRCA2* mutation carriers

Pinchas Osin, Tim Crook, Trevor Powles, Julian Peto, Barry Gusterson

The US National Cancer Institute (NCI) and the National Adjuvant Breast and Bowel Project (NSABP) have prematurely released data from the Breast Cancer Prevention Trial (BCPT) that shows a reduction in the incidence of breast cancer in all age-groups studied among women receiving tamoxifen (web site <http://www.nci.nih.gov>).

In our view, it would not be appropriate to terminate the similar randomised trials that are being done in the UK and Italy on the basis of these interim results. No data on survival are yet available, since there have been only three deaths from breast cancer in the tamoxifen group and five in the placebo group in BCPT. The eventual effects on endometrial cancer, pulmonary embolism, and deep-vein thrombosis, which together total 80 cases in the tamoxifen and 39 in the placebo group, are also unknown. The long-term balance of risks and benefits is even less clear for women who are carriers of mutations in the breast cancer susceptibility genes *BRCA1* or *BRCA2*.

Tumours arising in a mutant *BRCA1/BRCA2* background are of high grade,¹ are more likely to be oestrogen-receptor (ER) and progesterone-receptor (PgR) negative,² and also have a high incidence of *TP53* mutations.^{3,4}

Such tumours tend to be less responsive to tamoxifen than sporadic breast cancer, and randomised trials are essential to find out whether tamoxifen can prevent or delay the clinical presentation of these tumours and whether this will improve overall long-term survival.

To obtain some indication of the stage in tumour progression at which hormone independence may arise, we studied the phenotype of 40 invasive breast cancers arising in women who are germline mutation carriers for *BRCA1* (25 patients) or *BRCA2* (15). 16 of these tumours had associated non-invasive ductal carcinoma-in-situ (DCIS) and we have assessed the ER and PgR expression in this non-invasive component as a possible marker of endocrine resistance. In the invasive component, 35 of the 40 (87%) cancers were grade III with 67% ER-negative and 95% PgR-negative. In the 16 cases of associated DCIS (eight *BRCA1* and eight *BRCA2*) 11 (66%) were negative for ER and all 15 were negative for PgR (one case was not stained due to lack of material). All cases were scored using immunohistochemistry and the "quick-score" method, in which a score of 3 or above is taken as positive.⁵

This is a small group of tumours and may not be representative of tumours associated with *BRCA1* and *BRCA2* as a whole, but the results do indicate that a notable proportion of breast cancers arising in this background have a hormone resistance phenotype at the stage at which they are first morphologically recognisable as being malignant. This does not imply that tamoxifen cannot prevent these

tumours from arising, but does indicate the importance of clinical trials to assess this question.

TC is Leopold Muller Fellow, PO is supported through the Gilbert Fellowship. The study was supported by the Cancer Research Campaign and the Institute of Cancer Research. We thank B Ponder, D Easton, and M Stratton for access to clinical material and the familial cancer database.

- 1 Breast Cancer Linkage Consortium. The pathology of familial breast cancer: differences between breast cancer in carriers of *BRCA1* and *BRCA2* mutations and sporadic breast cancer. *Lancet* 1997; **349**: 1505–10.
- 2 Johannsson OT, Idvall I, Anderson C, et al. Tumour biological features of *BRCA1*-induced breast and ovarian cancer. *Eur J Cancer* 1997; **33**: 362–71.
- 3 Crook T, Crossland S, Crompton MR, Osin P, Gusterson BA. *p53* mutation in *BRCA1*-associated familial breast cancer. *Lancet* 1997; **350**: 638–39.
- 4 Gretarsdottir S, Thorlacius S, Valgardsdottir R, et al. *BRCA2* and *p53* mutations in primary breast cancer in relation to genetic instability. *Cancer Res* 1998; **58**: 859–62.
- 5 Detre S, Sacconi Jotti G, Dowsett MA. "Quick score" method for immunohistochemical semiquantitative validation for oestrogen receptor in breast carcinomas. *J Clin Pathol* 1995; **48**: 876–78.

Section of Cell Biology and Experimental Pathology (B A Gusterson), and Section of Epidemiology, Institute of Cancer Research, Haddow Laboratories, Sutton, Surrey SM2 5NG, UK

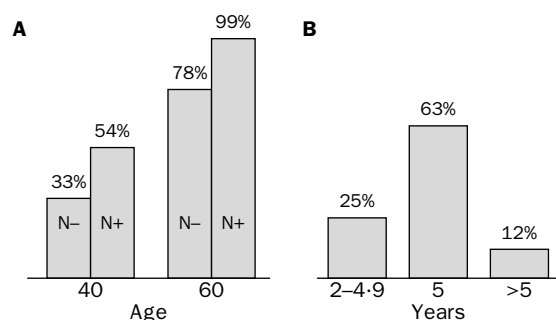
Variation in use of adjuvant tamoxifen

Christina Davies, Paul McGale, Richard Peto

In 1997, we asked about the prescription of adjuvant tamoxifen for breast cancer by medical opinion leaders in continental Europe, Asia, South America, and Australasia. We defined an opinion leader by attendance at international meetings or interest/participation in clinical trials. Our survey was not intended as a random sample of doctors or patients, but the qualitative findings are of substantial relevance.

Questionnaires were sent twice to 3000 doctors, asking whether they treated breast cancer and, if so, whether they usually used adjuvant tamoxifen in women aged 40 years (premenopausal) or 60 years (postmenopausal) with (N+) or without (N-) local lymph-node involvement. The questionnaire also asked how long they would usually continue such treatment. 1053 replied, including 841 who treat breast cancer. Of 1947 non-responding addressees, we telephoned a random sample of 50: 62% were doctors who treat breast cancer and could have replied.

Among those who treat breast cancer, the replies indicated wide variation in prescribing practice. However, the overall percentages with particular prescribing patterns were similar for respondents from different regions, for the randomly



Patterns of adjuvant tamoxifen use

A: Effect of age and lymph-node involvement on percentage of breast-cancer doctors who would routinely consider adjuvant tamoxifen.

B: Usual duration of any adjuvant tamoxifen (percentage of doctors, not patients).

telephoned non-respondents and for each of the different specialties dealing with breast cancer. Almost all these breast-cancer doctors (99%) would generally use tamoxifen in older women with N+ disease, but only half (54%) would do so in younger women with N+ disease. These percentages were smaller (78% and 33%, respectively) for women with node-negative disease (figure, A).

Although age and, to a lesser extent, nodal status strongly affect whether adjuvant tamoxifen is prescribed, they had little effect on the usual duration of the regimen used which, for 75% of these doctors, was at least 5 years (figure, B). 12% of clinicians used a longer regimen, however, indicating that a significant minority of these opinion leaders hope for additional benefit from continuing tamoxifen beyond the initial 5 years. That may be justified, but such continuation still needs reliable assessment.¹

As the evidence from the randomised trials of adjuvant tamoxifen continues to evolve,²⁻⁴ patterns of tamoxifen use will continue to change. The trials, as summarised by the most recent quinquennial worldwide review,⁴ now show that, at least for women with some oestrogen-receptor protein detectable on their primary tumour (and for women with no oestrogen-receptor assay done), about 5 years of tamoxifen substantially delays recurrence and improves 10-year survival. This is true not only for older women but also for younger women, irrespective of nodal status. Hence, if the general willingness to use tamoxifen for postmenopausal women with N+ disease were to be extended to those with N- disease, and to younger women, many more deaths could be avoided. Even for those who receive chemotherapy as part of their adjuvant treatment, addition of tamoxifen confers extra benefit.⁴

More than a million women worldwide are now prescribed tamoxifen, but the present heterogeneity in practice is disturbing, particularly with respect to the treatment of younger women (among whom only half of the respondents in our survey would use tamoxifen).

We thank the clinicians who responded. Helen Monaghan, Alison Naughten, and Abigail Headon coordinated the survey.

- 1 Peto R. Five years of tamoxifen—or more? *J Natl Cancer Inst* 1996; **88**: 1791–93.
- 2 Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of adjuvant tamoxifen and cytotoxic therapy on mortality in early breast cancer. *N Engl J Med* 1988; **319**: 1681–92.
- 3 Early Breast Cancer Trialists' Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy: 133 randomised trials involving 31 000 recurrences and 24 000 deaths among 75 000 women. *Lancet* 1992; **339**: 1–15, 71–85.
- 4 Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomised trials. *Lancet* 1998; **351**: 1451–67.

MRC/ICRF Clinical Trial Service Unit, University of Oxford, Radcliffe Infirmary, Oxford OX2 6HE, UK (C Davies)

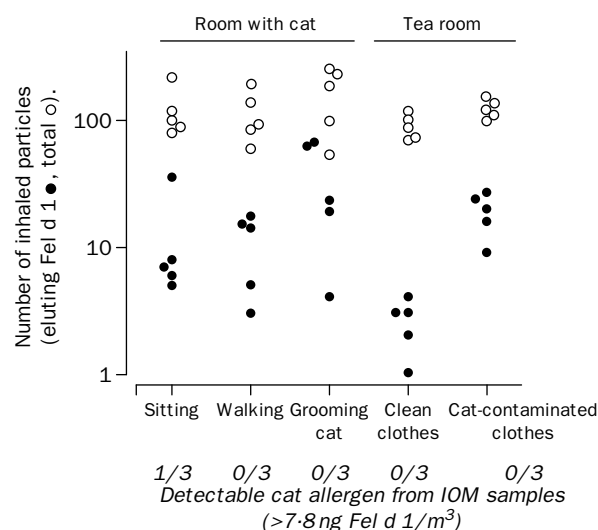
Detection of inhaled cat allergen

Timothy J O'Meara, Sandra De Lucca, Richard Sporik, Anthony Graham, Euan Tovey

Measuring personal exposure to airborne allergens is important in understanding the association between asthma and allergen exposure.¹ Personal exposure to cat allergen (Fel d 1) is likely to involve a more complex pattern of exposure than indicated by either reservoir dust or long-term air Fel d 1 concentrations.^{2,3} An additional limitation of current methods of air sampling is that the quantity of allergen recovered is low compared with the sensitivity of detection methods. We have developed a passive air sampler worn in each nostril which collects inhaled particles (mainly >5 µm) and a method that detects the allergen eluted from each particle.

Nasal air samplers were worn by the same individual for 10 min during five replicates of a range of exposure situations in a living room with a cat (31.7 µg Fel d 1 per g carpet dust, 569.6 µg Fel d 1 per g sofa dust) and in the low-allergen environment of a tea room in the University (2.8 µg Fel d 1 per g floor dust). In one sampler, inhaled particles were collected by impaction on to a protein-binding membrane and were immunostained with monoclonal antibodies specific to Fel d 1 (Indoor Biotechnologies, University of Virginia) to detect allergen localised around the inhaled particles. The total number of particles collected and the number of particles containing Fel d 1 were counted, and are expressed as medians. In the other sampler, particles were collected on a non-protein-binding membrane and the eluted allergen measured by ELISA.² The detection of Fel d 1 by these methods was compared with a standard personal air sampler (Institute of Occupational Medicine [IOM], Edinburgh) and eluted allergen detected by ELISA. 23 particles containing Fel d 1 were inhaled per nostril, in 10 min, while grooming a cat (figure).

In the University tea room, only three particles containing Fel d 1 were collected while wearing clean clothing, but this increased to 20 particles while wearing clothing previously contaminated by exposure to a cat (12.5 µg Fel d 1 per g of clothes dust). Cat allergen was detected on 3–19% of all inhaled particles, depending on the activity. By contrast, airborne Fel d 1 was only detected in samples eluted from the nasal filter during the high-exposure activity of cat grooming (three of five samples, maximum ~17.5 ng Fel d 1 per m³, assuming an inspiratory flow rate of 10 L/min), while walking in a room with a cat (two of five samples [figure], maximum ~33.3 ng/m³), and from the IOM



Inhaled particles (per nostril) containing cat allergen

sampler while sitting next to the cat (one of three samples, 32.5 ng/m³).

This system provides a sensitive method for the direct measurement of personal allergen exposure. It graphically confirms the importance of clothes as a source of cat allergen.^{4,5} Although it may underestimate the amount of inhaled allergen carried on particles smaller than 5 µm (~25% of cat allergen),² this system may be a useful method for measuring exposure not only to cat, but also to other allergens.

This project was funded by the Allergen Diagnostic Project Syndicate.

- 1 NAEPP/NHLBI. Expert Panel Report II: guidelines for the diagnosis and management of asthma. Bethesda, MD: National Institute of Health, No 97-4051, July, 1997.
- 2 Luczynska CM, Li Y, Chapman MD, Platts-Mills TAE. Airborne concentrations and particle size distributions of allergen derived from domestic cats (*Felis domesticus*): measurements using cascade impactor, liquid impinger and a two-site monoclonal antibody assay for Fel d 1. *Am Rev Respir Dis* 1990; **141**: 361–67.
- 3 de Blay F, Chapman MD, Platts-Mills TAE. Airborne cat allergen (Fel d 1): environmental control with the cat in situ. *Am Rev Respir Dis* 1991; **143**: 1334–39.
- 4 D'Amato G, Liccardi G, Russo M, Barber D, D'Amato M, Carreira J. Clothing is a carrier of cat allergens. *J Allergy Clin Immunol* 1997; **99**: 577–78.
- 5 Patchett K, Lewis S, Crane J, Fitzharris P. Cat allergen (Fel d 1) levels in primary school classrooms in Wellington, New Zealand. *J Allergy Clin Immunol* 1997; **100**: 755–59.

Institute of Respiratory Medicine, University of Sydney, New South Wales, Australia 2006 (T J O'Meara)

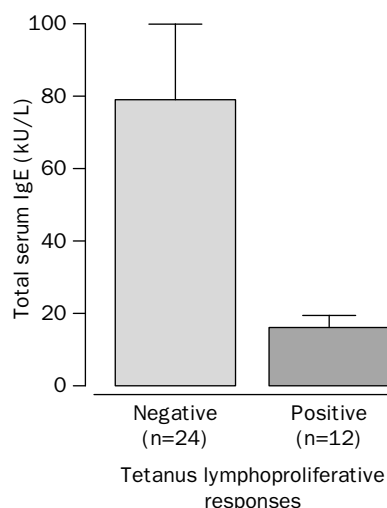
Raised serum IgE associated with reduced responsiveness to DPT vaccination during infancy

Susan L Prescott, Peter D Sly, Patrick G Holt

Th-2-skewed immunity against allergens in association with raised IgE antibody production is a hallmark of the atopic phenotype, and contrasts with the Th-1-like pattern of immunity expressed in non-atopics. Recent studies indicate that these differing responses are commonly programmed into immunological memory during early childhood, and represent the outcome of competition between mutually antagonistic IL-4-secreting Th-2 cells and IFNγ-secreting Th-1 cells which are activated by repeated allergen exposure.

On the basis of infant T-cell cloning studies,¹ we have proposed that delayed postnatal maturation of certain adaptive immune functions, particularly secretion of IFNγ, is a key determinant of genetic susceptibility to atopic disease—ie, this transient maturational deficiency in Th-1 function would favour the initial development of Th-2 polarised immunological memory. Support for this concept has come from various studies which showed an association between reduced capacity for IFNγ production and family or personal history of atopy, by tests of peripheral blood mononuclear cells from young children and also cord blood.² Additionally, a retrospective study of 12-year-old children³ showed that allergic disease was most common among those who had previously failed to develop Th-1-dependent delayed tuberculin hypersensitivity in response to BCG vaccination during infancy.

We report the results of parallel studies on serum IgE concentrations and responsiveness to the DPT vaccine, in a cohort of 49 children whose vaccination records could be validated; the children were sampled at 1 and 2 years of age. Cellular immunity to tetanus toxoid (TT) was measured by



Serum IgE concentrations of 2-year-olds who showed positive lymphoproliferation responses to tetanus toxoid (TT) compared with those who failed to show a response

Lymphoproliferation was assayed as incorporation of ³H-thymidine into DNA in the presence versus absence of TT, and expressed as disintegrations per min (dpm) above background; incorporation levels above 1000 dpm were taken as positive responses (detailed methods in ⁴).

lymphoproliferation. Total serum IgE and diphtheria-specific and tetanus-specific IgG were measured at each visit by ELISA.

The most striking finding in this cohort is the inverse relation between serum concentrations of IgE and in-vitro responses to TT vaccine antigen. The figure illustrates that 2-year-old infants who fail to develop proliferative responses to TT have higher serum IgE concentrations than their TT-responsive counterparts ($p=0.03$, Mann Whitney analysis). Spearman correlations showed that serum IgG value at 1 year were inversely related to in-vitro TT responses at 1 year ($r=-0.4$; $p=0.03$) and at 2 years ($r=-0.5$; $p=0.0004$). There was a strong correlation between IgE concentrations at 1 and 2 years of age ($r=0.7$, $p<0.0001$). Higher IgE was also associated with lower concentrations of diphtheria-specific IgG at 1 year ($r=0.4$, $p=0.03$) and 2 years ($r=-0.4$, $p=0.035$). Tetanus-specific IgG responses followed a similar pattern, which did not achieve significance ($p=0.08$) although levels were positively correlated with proliferative responses to TT ($r=0.4$, $p=0.01$).

These findings provide further support for the concept that the atopic phenotype is associated with a subtle underlying developmental defect in features of cellular immune function. It is not clear from these studies whether the magnitude of this deficiency is sufficient to compromise vaccine efficacy in this age group, but this issue could be addressed in retrospective surveys.

This work was supported by GlaxoWellcome UK and the NHMRC of Australia

- 1 Holt PG, Clough JB, Holt BJ, et al. Genetic "risk" for atopy is associated with delayed postnatal maturation of T cell competence. *Clin Exp Allergy* 1992; **22**: 1093.
- 2 Holt PG, Sly PD, Björkstén B. Atopic versus infectious disease in childhood: a question of balance? *Pediatr Allergy Immunol* 1997; **8**: 53.
- 3 Shirakawa T, Enomoto T, Shimazu S, Hopkin J. Inverse association between tuberculin responses and atopic disorder. *Science* 1997; **272**: 77.
- 4 Prescott SL, Macaubas C, Holt BJ, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T-cell responses towards Th-2 cytokine profile. *J Immunol* (in press).

TwW Telethon Institute for Child Health Research, PO Box 855, West Perth 6872, Western Australia (PG Holt); and Department of Paediatrics, University of Western Australia, Perth

Prevalence of tuberous sclerosis estimated by capture-recapture analysis

Finbar J K O'Callaghan, Alistair W Shiell, John P Osborne, Christopher N Martyn

Tuberous sclerosis is a dominantly inherited disease of high penetrance characterised pathologically by the presence of hamartomata in multiple-organ systems. Common clinical associations include epilepsy, learning difficulties, and behavioural problems. The disease is genetically heterogenous. Mutations in two recently identified genes, *TSC1* at 9q34¹ and *TSC2* at 16p13,² each result in a clinically indistinguishable phenotype. Estimates of the prevalence of tuberous sclerosis in the UK have ranged from 0.7 per 100 000 population in Northern Ireland³ to 3.8 per 100 000 population in Wessex.⁴

Accurate estimates of prevalence depend on completeness of case ascertainment. To achieve this, investigators usually try to locate cases from as many different sources as are available, eliminate duplicates, and calculate prevalence from the total. The implicit assumption that this approach identifies all cases is frequently unjustified. Epidemiologists have now realised that the capture-recapture methods used by animal ecologists for censuses of wildlife populations can be adapted for human populations. The underlying idea is simple. The size of a population can be estimated by trapping and marking a sample and then releasing the marked sample and allowing it to mix with the other members of the population, before trapping a second sample. The proportion of marked to unmarked individuals in the second catch is the same as the proportion of the number in the first catch to the number in the whole population. People, of course, are effectively marked already by their name and date of birth. So, where more than one source of information has been used to identify cases in a survey, the different sources may be thought of as different trappings and the number of cases that have been missed can be estimated from the overlap between sources. In this simple formulation, assumptions are made that each member of the population has an equal chance of being caught in any particular trapping and that being caught once does not influence the chance of being caught a second

time. Unfortunately, in human populations these assumptions are frequently violated. The problem can be minimised by the use of techniques such as log-linear modelling but a high degree of accuracy in the estimate of numbers of missing cases should not be expected.

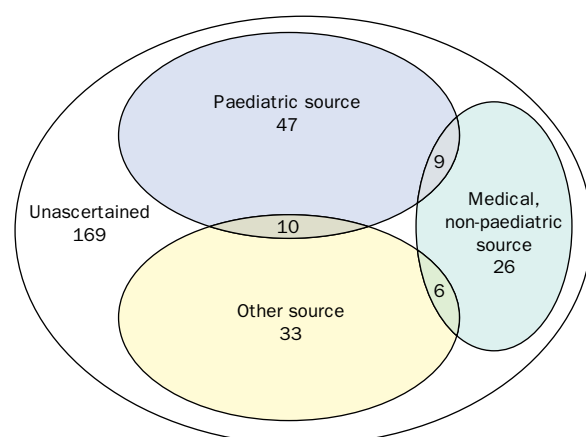
We carried out a capture-recapture analysis of data collected in the survey of prevalence of tuberous sclerosis in Wessex. Cases had been identified from a large number of different sources. Data from these individual sources were pooled to construct three summary sources: cases identified by paediatricians; cases identified by other medical practitioners; and cases identified from other sources (for example, the Tuberous Sclerosis Association or Hospital Activity Analysis). We used the method described by Hook and Regal⁵ to first derive estimates for the total number of cases from each of the three possible two-source models and finally from all three sources.

The figure shows the number of cases identified from each source and the overlap between sources. No cases were identified from all three sources. Maximum likelihood estimates are given for the total number of people with tuberous sclerosis in this population derived from each of the two-source models and from the three-source model. Dependency between sources was investigated by including terms for interaction between sources in the log-linear model; none of these terms was statistically significant.

The Wessex survey originally identified 131 cases of tuberous sclerosis in a population of 3.4 million. The results of the capture-recapture analysis suggest that, despite the efforts of the investigators to locate all cases, more than half remained undetected. A revised estimate of prevalence, taking account of unascertained cases, is 8.8 per 100 000 population (95% CI 6.8–12.4). One implication is that many people with tuberous sclerosis do not receive either genetic counselling or specialist medical supervision.

- 1 Van Slegtenhorst M, de Hoogt R, Hermans C, et al. Identification of the tuberous sclerosis gene *TSC1* on chromosome 9q34. *Science* 1997; 277: 805–08.
- 2 The European Chromosome 16 Tuberous Sclerosis Consortium. Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell* 1993; 75: 1305–15.
- 3 Stevenson AC, Fisher OD. Frequency of epiloia in Northern Ireland. *Br J Prev Soc Med* 1956; 10: 134–35.
- 4 Webb DW, Fryer AE, Osborne JP. Morbidity associated with tuberous sclerosis: a population study. *Dev Med Child Neurol* 1996; 38: 146–55.
- 5 Hook EB, Regal RR. Capture-recapture methods in epidemiology: methods and limitations. *Epidemiol Rev* 1995; 17: 243–64.

MRC Environmental Epidemiology Unit, University of Southampton, Southampton General Hospital, Southampton SO16 6YD, UK (C Martyn); and Department of Paediatrics, Royal United Hospital, Bath



Number of cases of tuberous sclerosis identified from each source

Total number of cases = 300.

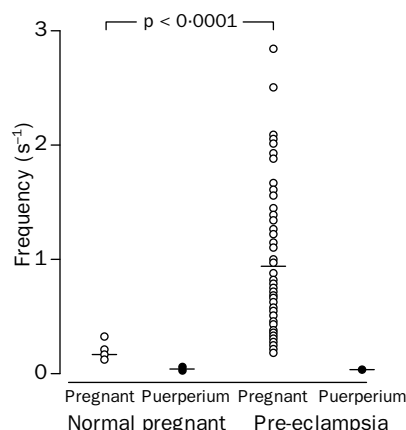
Maximum likelihood estimate for two-source models: paediatric versus non-paediatric 300.7 (95% CI 192–568); non-paediatric versus other 334.8 (189–775); paediatric versus other 323.4 (210–588). Maximum likelihood estimate for three-source model 300.3 (230–421).

CA⁺⁺ oscillation in endothelial cells and pre-eclampsia

Keiichi Matsubara, Hiroshi Ochi, Hiroyuki Kitagawa, Masaharu Ito, Keizo Oka

Because pre-eclampsia improves rapidly after delivery, it is thought to be caused by a disturbance in uteroplacental circulation. Injury to endothelial cells caused by serum may have a role in the onset of pre-eclampsia.¹ We investigated the relation between serum samples from pre-eclamptic patients and endothelial cell Ca⁺⁺.

Human umbilical vascular endothelial cells (HUVECs) were obtained from umbilical cords of normal deliveries² and cultured for 3–5 days. By incubation with HBSS/1%



Effect of serum samples from normal pregnant women and patients with pre-eclampsia on Ca^{++} oscillation in endothelial cells

Effect disappeared 1 week after delivery. Horizontal bars=mean values.

BSA including 10 $\mu\text{mol/L}$ fluo-3/AM for 1 h at 37°C in 5% CO_2 , fluo-3 was loaded into endothelial cells. After being washed twice, the supernatant was replaced by 1 mL of HBSS/1% BSA. Then 50 μL serum samples from eight non-pre-eclamptic pregnant women (29–37 gestational weeks), or six patients with pre-eclampsia (31–37 gestational weeks) were added to the culture medium, and the change of Ca^{++} in all HUVECs (normal=42 cells, pre-eclampsia=71 cells) within a field of view was measured by confocal laser scanning microscopy (ACAS 570, Meridian Instruments, Inc, Okemos, MI, USA). The measurements were consistent within 15%. Pre-eclampsia was defined as hypertension (systolic blood pressure of over 140 mm Hg, diastolic pressure of over 90 mm Hg, or both). Values are presented as the means (SD). Statistical analysis was with Fisher's exact probability test or Mann-Whitney U test.

The Ca^{++} oscillation induced by serum samples from pre-eclamptic patients was significantly higher than that of normal pregnant women (82% vs 19%, $p < 0.0005$) and the frequency of oscillation was significantly higher when compared with normal pregnant women ($0.95[0.62] \text{ min}^{-1}$ vs $0.19[0.06] \text{ min}^{-1}$, $p < 0.0001$). 1 week after delivery, the differences disappeared (figure).

As an intracellular second messenger, Ca^{++} is involved in cell proliferation and expression of cellular functions. Oscillation, which indicates a periodic regulation of Ca^{++} in cells, is induced by the repetition of the release and re-intake of Ca^{++} from and into the sarcoplasmic reticulum through a receptor reaction.³ Endothelial cells have an important part to play in maintaining the homeostasis of pregnancy: they regulate the intravascular coagulation system and the vascular tone, by producing anticoagulants, vasodepressor factors, and vasopressor factors. Sunano and colleagues⁴ reported that acetylcholine induced endothelial-cell-dependent periodic arterial constriction in normotensive rats. It is possible that the vasoconstriction is regulated by the periodic production of nitric oxide from endothelial cells, induced by Ca^{++} oscillation.⁵

We believe that some factors in serum samples from patients with pre-eclampsia do not have direct cytotoxic effects as has been assumed, but they could have a protective role by strongly stimulating periodic secretion of nitric oxide by endothelial cells in a hypertensive environment. Conversely, our study could also indicate that endothelial-cell injury in the placental circulation of patients with pre-eclampsia causes a compensatory increase of factors to stimulate the production of nitric oxide by endothelial cells.

- 1 Rodgers G, Taylor R, Roberts J. Pre-eclampsia is associated with a serum factor cytotoxic to human endothelial cells. *Am J Obstet Gynecol* 1988; **159**: 908–14.
- 2 Jaffe E, Nachman R, Becker C, Minick C. Culture of human endothelial cells derived from umbilical veins. *J Clin Invest* 1973; **52**: 2745–56.
- 3 Abdel-Latif A. Calcium-mobilizing receptors, polyphosphoinositides, and the generation of second messengers. *Pharmacol Rev* 1986; **38**: 227–72.
- 4 Sunano S, Sasaki F, Osugi S, Shimamura K. Comparison of endothelium dependent and independent tension oscillation in aortae of stroke-prone spontaneously hypertensive rats and Wistar Kyoto rats. *J Smooth Muscle Res* 1994; **30**: 135–45.
- 5 Gustafsson H. Vasomotion and underlying mechanisms in small arteries. An in vitro study of rat blood vessels. *Acta Physiol Scand Suppl* 1993; **614**: 1–44.

Department of Obstetrics and Gynecology, Ehime University, School of Medicine, Shigenobu-cho, Onsen-gun Ehime, 791-0295, Japan

Exhaled nitric oxide and pulmonary response to iloprost in systemic sclerosis with pulmonary hypertension

Giovanni Rolla, Paola Colagrande, Luisa Brussino, Caterina Bucca, Maria Tiziana Bertero, Federico Caligaris-Cappio

Widespread endothelial damage occurs in patients with systemic sclerosis, a disease frequently complicated by increased pulmonary artery resistance.¹ The synthesis and release of nitric oxide (NO) are thought to be impaired in these patients. NO is produced by various cells in the lower respiratory tract, including pulmonary vascular endothelial cells, and can be measured in expired air.² A decreased NO concentration in the exhaled air of patients with systemic sclerosis and pulmonary hypertension has been reported.³ Although aerosol administration of iloprost, a stable analogue of prostaglandin, is increasingly used in the treatment of pulmonary hypertension associated with collagen disease,⁴ the responsiveness of the pulmonary vasculature may not be uniform among patients. In this respect, the presence or absence of structural and functional changes in lung vessels may be important. Measurement of exhaled NO after pharmacological stimuli could represent a non-invasive method to functionally evaluate pulmonary endothelium and, possibly, to identify which patients are more responsive to vasodilating drugs.

Two women aged 53 (patient 1) and 57 (patient 2) years with systemic sclerosis and pulmonary hypertension (pulmonary artery pressure 56 and 58 mm Hg, respectively) gave their informed consent to be studied. Both women had mild-to-moderate interstitial fibrosing alveolitis quantified by thin-section computerized tomography. The diagnosis was made, respectively, 4 and 5 years earlier; current therapy consisted of low-dose corticosteroids. Both patients had moderate restrictive lung impairment (total lung capacity 73 and 68% of predicted), with marked decrease in carbon monoxide diffusing capacity adjusted by alveolar volume (K_{co} 42 and 37% of predicted), normal airway patency ($\text{FEV}_1/\text{VC} \times 100$, 84 and 80%), and moderate hypoxemia (PaO_2 65 and 67 mm Hg, respectively). Mean baseline exhaled NO was 2.8 (SE 0.84) and 3.4 (1.14) (mean values of five sets of measurements, obtained in different days)—ie, lower than our reference normal value (5.75 [1.6] ppb). 30 min after intravenous administration of L-arginine (NO precursor) (0.5 g/kg) and, on a separate day, during 20 min glyceryl trinitrate infusion (33 $\mu\text{g/min}$), patient 1 showed an increase in exhaled NO, reaching 6.3 and 8.2 ppb, respectively, starting from 2.4 and 3.1 ppb after

intravenous saline administration. No change in exhaled NO was observed in patient 2 during the same infusion tests. No change in pulmonary artery pressure (PAP), measured by doppler echocardiography, occurred after L-arginine or during glyceryl trinitrate infusion. Both patients received sequentially aerosolised saline and iloprost (20 µg in saline 4 mL), after measurement of baseline exhaled NO, PaO₂, and pulmonary artery pressure. 30 min after iloprost aerosol, patient 1 showed a brisk increase in exhaled NO (from 2.2 to 9.5 ppb) and PaO₂ (from 65 to 80 mm Hg), along with a decrease in pulmonary artery pressure (from 54 to 29 mm Hg), whereas no change was observed in patient 2. No significant change in heart rate or in systemic arterial blood pressure was observed throughout the experiment.

These data confirm the previously reported³ low baseline exhaled NO concentration in patients with systemic sclerosis and pulmonary hypertension. The increase of exhaled NO after pharmacological stimuli was related to the favourable response of pulmonary vasculature to the vasodilator iloprost. The lack of increase of exhaled NO was associated with a lack of effect of iloprost on pulmonary artery pressure. It is unlikely that pulmonary vasodilation of iloprost in patient 1 depends only on the increase in exhaled NO, because a similar increase of exhaled NO after L-arginine and glyceryl trinitrate was not followed by a decrease in pulmonary artery pressure. In isolated perfused lungs of rats, the relaxant effect of iloprost on vascular smooth muscle is mediated in part directly by K_{ATP} channels and K_{Ca} channels and in part by NO release.⁵

Because of the favourable response to inhaled iloprost, long-term therapy with iloprost (100 µg daily in five aerosols) was initiated in patient 1. Daily inhalation of iloprost was well tolerated. Sustained responsiveness in terms of pulmonary artery pressure, PaO₂, and exhaled NO has been observed over a 3-month period. The patient's dyspnoea on effort decreased, with a clinically relevant increase in the 6 min walking test, from 285 m to 510 m, and an increase in K_{Co} from 42% to 55% of predicted.

Although limited to two patients, our observation suggests that exhaled NO measurement after pharmacological stimuli may give important information on the responsiveness of the pulmonary circulation and might develop as a simple test to identify which patients with systemic sclerosis and pulmonary hypertension are likely to be responsive to vasodilating drugs. Further studies with different vasodilators, including NO itself, are needed.

Supported by grants from Ministero dell'Università e della Ricerca Scientifica e Tecnologica and from PFCNR Biotechnologie.

- 1 Ungerer RG, Tashkin DP, Furst D, et al. Prevalence and clinical correlates of pulmonary artery hypertension in progressive systemic sclerosis. *Am J Med* 1983; **75**: 65–74.
- 2 Gustafsson LE, Leone AM, Persson MG, Wiklund NP, Moncada S. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea-pigs and humans. *Biochem Biophys Res Commun* 1991; **181**: 852–57.
- 3 Cailles JB, Kharitonov S, Yates D, Barnes P, Dubois RM. Decreased endogenous nitric oxide in the exhaled air of systemic sclerosis patients with pulmonary hypertension. *Thorax* 1995; **50**: 452P (abstr).
- 4 Olschewski H, Walrmath D, Schermuly R, Ghofrani A, Grimminger F, Seeger W. Aerosolized prostacyclin and iloprost in severe pulmonary hypertension. *Ann Intern Med* 1996; **124**: 820–24.
- 5 Dumas M, Dumas J, Rochette L, Advenier C, Giudicelli JF. Role of potassium channels and nitric oxide in the effects of iloprost and prostaglandin E₁ on hypoxic vasoconstriction in the isolated perfused lung of the rat. *Br J Pharmacol* 1997; **120**: 405–10.

Dipartimento di Scienze Biomediche e Oncologia Umana, University of Torino, 10126 Torino, Italy (G Rolla); and Divisione Universitaria di Immunologia Clinica e Allergologia, Ospedale Mauriziano Umberto I, Torino e Laboratorio di Immunologia Oncologica, IRCC, Candiolo

Cold hands, warm heart

Han Kim, Clark Richardson, Jeanette Roberts, Lisa Gren, Joseph L Lyon

The saying "cold hands, warm heart" probably has a basis in temperature differences between men and women. In medicine, infrared tympanic thermometers (ITT) are widely used to quickly and reliably determine core temperature by measuring the infrared radiation given off by the tympanic membrane.^{1,2} This technology can also be applied to measure the temperature on any body surface and has been successfully used to measure the temperature of the cornea.³ We used this instrument to investigate the role of sex in determining core and hand temperatures.

We studied 219 people whose ages ranged from under 1 year to 84 years (78 male, 141 female). Each completed a questionnaire, reporting date of birth, sex, height, weight, menstrual status, and stage of menstrual cycle. The ITT used was a First Temp Genius (Sherwood-Davis & Geck, St Louis, Missouri, USA). Measurements were taken on the fingernails of the left and right middle fingers, and on the left and right tympanic membranes. The ITT produced reproducible measurements. We obtained 20 repeat measures on 20 people. The average standard deviation in core temperature and hand temperature for these measurements was 0.23°F. A literature search found studies which concluded that ITTs do not measure core temperatures,^{4,5} as well as studies concluding that they did.¹

Women had a mean core temperature of 97.8 (SD 0.8)°F, whereas the mean core temperature for men was 97.4 (0.8)°F. The mean hand temperature for women was 87.2 (6.7)°F, compared with 90.0 (5.5)°F for men.

Menstrual status affected women's body temperatures. Postmenstrual women older than 50 years had significantly lower hand and core temperatures than premenstrual women below the age of 13 (OR 0.4 for both core and hand). Women in the first 14 days of their cycle were more likely to have lower core temperatures (OR=6.6) than women in the last 14 days.

Body-mass index (BMI) was also related to core temperature, but only in men. Men with higher BMI had lower core temperatures (OR 0.4). BMI had little effect on hand temperature. We also investigated the relation between core and hand temperature. We found that women with lower core temperatures were more likely to have lower hand temperatures (OR 3.4). We did not find this relation among men.

We found that women are more likely to have cold hands than men. This difference seems to be independent of age, BMI, and core temperature. However, women's core temperatures were on average 0.4°F (about 0.2°C) higher than men's. Thus, the adage "cold hands, warm heart" is based on real differences in temperature.

- 1 Edge G, Morgan M. The Genius infrared thermometer. *Anaesthesia* 1993; **48**: 604–07.
- 2 Yeo SA, Hayashi RH, Wan JY, Dubler B. Tympanic versus rectal thermometry in pregnant women. *J Obstet Gynecol Neonat Nurse* 1995; **24**: 719–24.
- 3 Fujishima H, Toda I, Yamada M, Sato N, Tsubota K. Corneal temperature in patients with dry eye evaluated by infrared radiation thermometry. *Br J Ophthalmol* 1996; **80**: 29–32.
- 4 Giuffre M, Fletcher J, Gravatt R, Hindt L, Ivey G, Hickman R. Investigating the reliability and validity of the infrared tympanic thermometer: a learning experience. *J Post-Anesth Nurs* 1995; **10**: 280–85.
- 5 Freed GL, Fraley JK. Lack of agreement of tympanic membrane temperature assessments with conventional methods in a private practice setting. *Pediatrics* 1992; **89**: 384–86.

Department of Family and Preventive Medicine, School of Medicine, University of Utah, Salt Lake City, Utah 84312, USA (H Kim)