

Abstracts

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Inishowen Gateway Hotel, Buncrana, Ireland



### S1. The Effects Of Obesity And Smoking On The Function Of Natural Killer Cells In Psoriasis

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Obesity has emerged as a major co-morbidity in patients with psoriasis. In a case control study we found that patients with psoriasis had significantly higher body mass indices (BMI) compared to controls ( $p = 0.004$ ). Increased adiposity and weight gain were strong risk factors for the development of psoriasis in the Nurses Health study<sup>1</sup>. Multivariate analysis demonstrated a clear, graded association between BMI and the risk of having psoriasis<sup>1</sup>.

Adipose tissue actively secretes cytokines such as TNF-alpha and interleukin-6 and pro-inflammatory adipokines such as leptin and resistin along with adiponectin which has anti-inflammatory effects. Patients with psoriasis had raised levels of serum resistin compared to controls<sup>2</sup>. Serum levels were also correlated with psoriasis disease activity and were unrelated to BMI suggesting a link between psoriasis and adipokines. A second study of 39 patients found disease severity was correlated with insulin resistance and serum resistin.

Recent studies have shown that patients who are obese have lower levels of natural killer (NK) cells when compared to controls. Natural killer cells from obese patients also had decreased levels of in-vitro activity compared to lean controls. Natural killer cells are thought to be involved in the pathogenesis of psoriasis and patients have lower levels of circulating NK cells than controls.

We have investigated levels of circulating NK cells in obese and lean patients with psoriasis, their in vitro cytotoxicity and also the effect of the adipokines leptin, adiponectin, resistin and cigarette smoke extract on the cytotoxicity of circulating NK cells.

CD 56; a marker of NK and NK-T cells; cells have been extracted from peripheral blood monocytes (PBMCs) of patients with psoriasis with magnetic beads. Using ethidium bromide and UV microscopy these CD 56 cells were counted. Psoriasis patients who are obese, defined as BMI > 30 (N = 6) have on average 3.84% CD 56 of total PBMCs cells versus patients who are lean defined as BMI < 25 (N = 7) have on average 8.9% CD56 cells ( $p = 0.02$ ).

CD 56 cells isolated above from PBMCs of patients with psoriasis and also controls were incubated with the myelogenous tumour line K562 in the presence of the adipokines resistin, leptin and adiponectin and also cigarette smoke extract for four hours. Using 7-AAD staining, the numbers of killed tumour cells were counted by flow cytometry and gating on the tumour cells.

In the presence of resistin tumour killing by CD 56 cells was increased by on average 28% when compared to CD 56 cells alone in six patients with psoriasis ( $p = 0.08$ ). In the presence of cigarette smoke extract the killing of CD 56 cells was decreased by on average 38 % in eleven patients with psoriasis ( $p = 0.001$ ).

These results indicate a possible mechanism whereby obesity affects psoriasis by further lowering NK cells. They also indicate that resistin and smoking may exacerbate this NK defect. By stimulating NK cells resistin may be hastening their apoptosis and cigarette smoke appears to be having a directly toxic effect.

1. Arathi RS, Curhan G, Hyon CK. *Arch Intern Med* 2007;176:13-27.
2. Johnston A, Arnadottir S, Gudjonsson, et al. *Br J Dermatol* 2008;159:342-350.

### S2. An audit to evaluate service outcome in children with Atopic Eczema attending a paediatric dermatology clinic.

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A re-audit was conducted to determine if the service provided at a paediatric dermatology out-patient clinic met the working standards for the management of atopic eczema as defined by the BAD. The re-audit results were compared to the results from the BAD Audit of atopic eczema management in secondary care, Phase 3: Audit of Service outcome, published in 2000. This audit was a prospective study of patients quality of life and disability preconsultation and 6 weeks postconsultation. Standards set were:

1. Of patients reporting sleep loss secondary to eczema, at least 70% should report a favourable change at 6 weeks.
2. There should be a 25% relative improvement in the CDLQI in 60% of patients within 6 weeks.
3. The management process should improve the worst aspect of the disease for 80% of patients.

4. Of those patients who are away from school because of their atopic eczema, 80% should return to school within 6 weeks following their initial assessment.

Standards were evaluated using 2 questionnaires offered to all new patients with a diagnosis of atopic eczema attending paediatric dermatology clinics from January-June 2008. Questionnaire 1 was completed at baseline. Questionnaire 2 was sent by post to the part 1 recruits 6 weeks after their initial consultations.

42 completed Part 1 and 27 completed Part 2 questionnaires were collected, amounting to an overall 64% response rate versus 67% response rate observed in the original audit.

Improvement in sleep loss at 6 weeks was attained in 52% of cases (standard set 70%). There was a >25% relative improvement in CDLQI score in 56% of patients falling short of the 60% standard set. Improvement in the worst aspect of disease was achieved in 67% cases, again not attaining the 80% standard set.

The 2008 re-audit results reveal more favourable outcomes compared to the original results in 2000, although fall short of the standards set. Are original working standards set too high? Is 6 weeks the optimum time to measure improvement in outcome? If non-responders did not reply because they had improved, the working standards may have been achieved.

Several recommendations have arisen following review of these results. These include inviting all new patients to complete a CDLQI; to identify for each individual what is the most troublesome aspect of their disease and re-evaluate at follow up and finally to avail of the input of a paediatric dermatology nurse specialist for all patients

### S3. Merkel Cell Polyomavirus (MCV) – a new skin cancer virus.

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Merkel cell carcinoma (MCC) is a rare and aggressive human skin cancer of neuroendocrine origin, with a high mortality and an increasing incidence worldwide. It predominantly affects elderly and immunocompromised individuals, a feature suggestive of an infectious origin. In early 2008, Feng *et al* described the presence of a novel virus in Merkel cell carcinomas (MCC)<sup>1</sup>. Viral DNA was integrated within tumour genome in a clonal pattern in 8 out of 10 MCC samples, suggesting that MCV infection and clonal integration may be the contributing factor in the pathogenesis of MCC. Another study compared MCC tumours from an American cohort of MCC to an Australian cohort, which demonstrated a much higher prevalence of the virus in the American cohort, 69% vs. 24%<sup>2,3</sup>. The regional difference suggests that there may be variable contributions of UV radiation and MCV to oncogenesis.

Our aim was to evaluate the percentage prevalence of MCV in MCCs to further substantiate the above findings, in our Irish cohort. We identified 7 patients who had Merkel cell carcinoma, 3 of whom were renal transplant recipients. Nucleic acid was extracted from the MCC tumour samples

and real-time and end point PCR was carried out to determine the percentage positivity.

Results showed 29% positivity in the detection of MCV in these tumours, in keeping with the findings of the Australian cohort. Deletion of viral elements and/or disruption of human tumour suppressor genes could conceivably contribute to uncontrolled cell growth. The highly related polyomavirus SV40 large tumour antigen is known to bind p53 and pRb which are both tumour suppressors and thus contribute to tumorigenesis by cell cycle deregulation. The two previously characterized MCV integration events by Fend *et al* lead to disruption these tumour suppressor regions in the MCV genome.

The role of MCV in the pathogenesis of MCCs of the skin and other neuroendocrine tumours has yet to be fully elucidated, but ongoing studies should provide new evidence in the near future.

1. Feng H, Shuda M, Chang Y, Moore PS Clonal Integration of Polyomavirus in Human merkel Cell Carcinoma. *Science* 2008;**319**(5866):1096-1100.
2. Kassem A, Schöpflin A, Diaz C, Weyers W, Stickeler E, Werner M, Zur Hausen A. Frequent Detection of Merkel Cell Polyomavirus in Human Merkel Cell Carcinomas and Identification of a unique Deletion in the VP1 Gene. *Cancer Res* 2008;**68**(13):5009-13.
3. Garneski KM, DeCaprio JA, Nghiem PJ. Merkel Cell Polyomavirus Is More Frequently Present in North American than Australian Merkel Cell Carcinoma Tumors. *Invest Dermatol* 2008.

### S4. Aberrant DNA methylation is linked with MTHFR C677T genetic polymorphism in cutaneous SCC.

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Changes in genomic DNA methylation associated with cancer include global DNA hypomethylation and gene specific hypermethylation. We have recently identified a genetic variant in the MTHFR gene involved in the methylation pathway which confers risk for the development of squamous cell carcinoma in renal transplant patients<sup>1</sup>. This genetic variant has also been discovered to confer risk in non transplant patients with low folate status<sup>2</sup>. Our aim in this study was to evaluate global and gene specific methylation status in SCC and non neoplastic skin in renal transplant patients and correlate this to the MTHFR polymorphism. We evaluated 87 skin samples in this study, including SCC and adjacent non neoplastic skin from 33 patients. Seventeen patients had the MTHFR polymorphism, 16 had no MTHFR polymorphism. SCC and non neoplastic skin were microdissected from paraffin blocks. DNA was extracted. PCR was carried out for specific genes p16 and MGMT and also LINE 1 which reflects global methylation. Quantitative evaluation of methylation levels was carried out by pyrosequencing after bisulphite modification of samples. Methylation analysis was evaluable in 40 SCCs and 36 non neoplastic skin samples. Global hypomethylation was evident in SCCs compared to adjacent non neoplastic skin (p<0.04). Patients with the MTHFR polymorphism had higher levels of methylation in tumours and non neoplastic skin compared to those without the MTHFR polymorphism (p < 0.002). Global hypomethylation appears to be a feature of SCC. Aberrant

methylation of DNA appears related to polymorphisms of MTHFR. In this study we found a functional effect of the MTHFR polymorphism significantly related to methylation of DNA. This might indicate that patients with the MTHFR polymorphism have an overall dysregulation of methylation in the genome of cancer and non neoplastic tissue. Together these findings suggest that intervention in the form of demethylating agents or folate supplementation may be beneficial in the treatment or prevention of SCC .

1. Laing ME *et al.* Association of methylenetetrahydrofolate reductase polymorphism and the risk of squamous cell carcinoma in renal transplant patients. *Transplantation* 2007;**84**(1):113-6. 2. Han J *et al.* Polymorphisms in the MTHFR and VDR genes and skin cancer risk. *Carcinogenesis* 2007;**28**(2):390.

### S5. The effect of isotretinoin on depressive symptoms in patients with acne vulgaris – a prospective cohort study 1999-2007

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Oral isotretinoin is an effective treatment for severe acne unresponsive to conventional therapies. Concerns remain over the drug’s safety profile and in particular its effect on mood status.

We designed a prospective cohort study to investigate the role of isotretinoin on mood status in patients with acne. Consenting patients initiating isotretinoin therapy completed a pre-treatment generic questionnaire and a modified Beck Depression Inventory (BDI) at week 0, 4, 16, and 4 weeks post-treatment. The BDI was composed of twenty-one

questions, each answer being a score of 0-3, depending on severity. All patients were treated with 0.5-1mg/kg of isotretinoin per day in two divided doses for 16-20 weeks.

486 patients were deemed eligible for inclusion, 58% male, with a mean (standard deviation) age of 21 (5.9). The overall trend was a decrease in BDI scores, with significant differences at all stages of treatment. Compared to baseline, the mean change in BDI was -0.43 (p=0.02) at week 4 (n=474), -0.628 (p=0.003) at week 16 (n=341), and -0.945 (p<0.0001) 4 weeks post-treatment (n=183). No increased risk of depression was identified in those individuals with baseline depressive symptoms (indicated by a baseline BDI ≥10), or a documented personal/family history of depression.

A review of medical notes and pharmacy records revealed that 2 of the 486 patients (0.4%) discontinued isotretinoin after developing mild depressive symptoms. Both reported an improvement in mood status following cessation of isotretinoin. There were no reports of deliberate self-harm, attempted or completed suicide, during treatment or follow-up. A further patient discontinued treatment in response to rectal bleeding.

As far as we are aware, this is the largest prospective study investigating the effect of isotretinoin on depressive symptoms. No evidence of a causal relationship between isotretinoin use and depression was identified. Mild depressive symptoms occurring in <1% of patients may reflect an idiosyncratic side-effect or be unrelated to isotretinoin.

It is difficult to conclusively exclude a link between isotretinoin and depression due to the confounding influence of acne and the risk of depression among the general population. Utilising a depression score such as the BDI when prescribing isotretinoin encourages dermatologists to monitor depressive symptoms, regardless of whether there is a causal relationship to the drug or not.