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The Effects of Acupuncture on Mucosal Immunity in the Upper Respiratory Tract

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DECLARATION

I hereby certify that the work embodied in this thesis is the result of original research. This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

John McDonald

DEDICATION

To my mother who taught me the value of education and hard work.

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Appendix A: McDonald, J.L., Cripps, A.W., Smith, P.K., Smith, C.A., Xue, C.C., & Golianu, B. (2013): The anti-inflammatory effects of acupuncture and their relevance to allergic rhinitis: A narrative review and proposed model. Evidence-Based Complementary and Alternative Medicine Article ID 591976. 158

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Chapter 1: Background

1.1 Abstract:

Allergic rhinitis is a common disease in Australia, with an estimated 3.17 million Australians affected. Allergic rhinitis represents a significant burden to the community in quality of life and wellbeing, impaired performance, loss of productivity and health care costs.

A review was undertaken firstly of the research literature on the efficacy and effectiveness of acupuncture for allergic rhinitis, then secondly of the research into the mechanisms of acupuncture in allergic rhinitis.

A total of 4 systematic reviews, 15 randomised controlled trials (RCTs) and other non-randomised studies on acupuncture treatment for allergic rhinitis were reviewed. Acupuncture was reported to significantly reduce signs and symptoms of allergic rhinitis in both children and adults. Evidence of acupuncture efficacy for persistent (perennial) allergic rhinitis was reported to be stronger than for intermittent (seasonal) allergic rhinitis.

Multiple physiological pathways appear to mediate the anti-inflammatory effects of acupuncture including the hypothalamic-pituitary-arenal (HPA) axis, sympathetic pathways, descending inhibitory pathways and possibly parasympathetic cholinergic pathways. Studies have also suggested that acupuncture may down-regulate pro-inflammatory neuropeptides and neurotrophins and may shift the Th1/Th2 balance in T helper cells and hence alter allergic status, however the evidence for these actions is inconclusive.

The primary objective of this study was to investigate whether or not acupuncture treatment produces modulation of the mucosal immune response in the upper respiratory tract in adults with allergic rhinitis. Hypothesised evidence of mucosal immune modulation would include down-regulation of neurotrophins and pro-inflammatory neuropeptides and a shift in the Th1/Th2 balance in T helper cells away from Th2 dominance.

The secondary objective was to investigate whether or not there was any reduction in markers of mucosal inflammation (such as nasal airway resistance, ECP and improved symptom and quality of life scores) following acupuncture.

The majority of previous studies have used exclusively subjective measures such as symptom and quality of life questionnaires. By measuring and comparing objective and subjective outcomes, this study aimed to provide more robust evidence for the effectiveness and efficacy of acupuncture treatment for allergic rhinitis. This study also sought to elucidate the precise nature of any mucosal modulation which might occur in response to acupuncture, contributing to a better understanding of the anti-inflammatory effects of acupuncture.

1.2 Introduction

There is evidence to suggest that acupuncture can modulate both non-specific and specific immunity. Published literature suggests that this modulation is most prominent in subjects with chronic inflammatory diseases such as allergic rhinitis. Allergic rhinitis is a significant burden to the Australian community in quality of life and wellbeing, impaired performance, loss of productivity and health care costs. The total number of Australians who have allergic rhinitis is estimated to be in the order of 3.7 million. While there is evidence that acupuncture treatment is clinically beneficial for patients with allergic rhinitis, little is currently understood about the mechanisms of acupuncture in this or other chronic inflammatory diseases which involve changes in the mucosal immune response. In this chapter the research on the clinical efficacy and effectiveness of acupuncture for the treatment of allergic rhinitis will first be reviewed, followed by a review of research into the mechanisms of acupuncture in modulating immune status with emphasis on anti-inflammatory actions, and specifically on changes in mucosal immunity in allergic rhinitis. Finally the proposed research plan and study design will be detailed.

The proposed study was designed to test the hypothesis that, in adult humans with allergic rhinitis, acupuncture down-regulates certain pro-inflammatory neuropeptides and neurotrophins as well as Th2 cytokines and pro-inflammatory cytokines, thereby producing a shift in the Th1/Th2 balance of T helper cells. Together these hypothesised actions would be expected to decrease clinical signs and symptoms of allergic rhinitis.

1.3 What is allergic rhinitis?

While the term “rhinitis” implies inflammation of nasal mucus membranes, clinically rhinitis can refer to any nasal disorder which includes any one or more of the symptoms: sneezing, nasal pruritus (itching), rhinorrhoea (runny nose) and nasal congestion (Wallace et al., 2008).

Rhinitis can be allergic or non-allergic, however allergic rhinitis is the most common form of chronic rhinitis (Dykewicz and Hamilos, 2010). Non-allergic rhinitis includes vasomotor rhinitis, gustatory rhinitis (rhinitis from foods and alcohol), infectious rhinitis, nonallergic rhinitis with eosinophilia syndrome (NARES), hormonal rhinitis, occupational rhinitis, drug-induced rhinitis, atrophic rhinitis and rhinitis associated with inflammatory-immunologic disorders (Wallace et al., 2008).

Allergic rhinitis refers to rhinitis which is triggered by contact with an inhaled allergen, however many patients with allergic rhinitis react to triggers which are not allergens such as cold air, perfumes and smoke. The number of patients who may have a mixture of allergic and non-allergic rhinitis has been estimated at between 44% and 87% of rhinitis patients (Wallace et al., 2008).

Links between allergic rhinitis and asthma have been highlighted by the ARIA group (an acronym for Allergic Rhinitis and its Impact on Asthma) (Bousquet et al., 2008; Braunstahl, 2009; Togias, 2003). Allergic rhinitis, which responds to seasonal triggers such as exposure to seasonal pollens, has been called “seasonal allergic rhinitis” (SAR), however in 2002 the ARIA group proposed that this term be replaced with “intermittent allergic rhinitis”(IAR)(Bousquet et al., 2008). The use of both terms persists in the current research literature (Wallace et al., 2008). Year-round allergic rhinitis has similarly been called “perennial allergic rhinitis” (PAR); likewise, the use of this term persists alongside the ARIA term “persistent allergic rhinitis” (also PAR) (Wallace et al., 2008). The ARIA group recommend that allergic rhinitis and asthma should be managed using a “united airway approach” (Bousquet et al., 2008; Braunstahl, 2009; Togias, 2003).

Rhinitis is frequently accompanied by symptoms of the eyes (such as conjunctivitis), ears and throat (Wallace et al., 2008).

1.3.1 Physiological and immune mechanisms of allergic rhinitis

Allergic rhinitis is caused by an allergic inflammatory response in the nasal mucosa triggered by exposure to an airborne allergen. This immunoglobulin-E(IgE)-mediated reaction involves a complex interaction between inflammatory cells such as eosinophils and mast cells, inflammatory cytokines which they release, pro-inflammatory neuropeptides which promote vasodilation and plasma extravasation and neurotrophins which prolong survival of inflammatory cells and contribute to hypersensitivity (Dykewicz and Hamilos, 2010). Disruption of the integrity of the nasal epithelium through cleaving of tight junctions by protease activities (due to inflammation or airborne allergens) exposes sensory nerve endings which enhances the neurogenic inflammatory response, especially the release of substance P (SP) and calcitonin gene-related peptide (CGRP) (Dykewicz and Hamilos, 2010; Finger et al., 1990).
The early-phase allergic response in allergic rhinitis is triggered within minutes of allergen inhalation when IgE antibody, bound to mast cells, recognises allergens and causes degranulation and release of inflammatory mediators such as histamine, tryptase, leukotrienes, prostaglandin D_2 and pro-inflammatory cytokines such as tumour necrosis factor alpha (TNFα) and interleukin 4 (IL-4) (Dykewicz and Hamilos, 2010). This early-phase response is generally characterised by sneezing, nasal itching and rhinorrhoea (runny nose) (Dykewicz and Hamilos, 2010; Wallace et al., 2008). Sneezing and nasal itching have been shown to be neural responses mediated by histamine (derived from mast cells) activating the histamine receptor H1R and the transient receptor potential vallinoid 1 (TRPV1) (Kim and Baraniuk, 2007; Shim and Oh, 2008; White, 1992). Rhinorrhoea is primarily a glandular response involving nasal epithelial cells, but also has neural and vascular contributions (Sarin, 2006; White, 1992). Plasma extravasation and vasodilation caused by mediators such as leukotrienes, prostaglandin D_2, nitric oxide and pro-inflammatory neuropeptides such as SP, CGRP and vasoactive intestinal peptide (VIP) create nasal congestion (Dykewicz and Hamilos, 2010; Lund, 1996). Kaise et al found that in guinea pigs, SP and CGRP released from nasal sensory nerves, possibly stimulated by mast cell-derived histamine, partially mediates the early-phase response (Kaise, 2001). The neurotrophin nerve growth factor (NGF) has been shown to contribute to early-phase response in allergic airway response but not to late phase response in rats with allergic asthma (Glaab, 2003).

Late-phase allergic response occurs 4 to 8 hours after the initial early-phase response as cytokines and other inflammatory mediators set off a cascade of events which switches the phenotype of B lymphocytes to plasma cells which produce specific IgE antibody and promotes the proliferation of mast cells and infiltration by eosinophils into the nasal epithelium and mucosa (Dykewicz and Hamilos, 2010). The symptoms of late-phase response are similar to those of early-phase response but with a greater predominance of nasal congestion (Dykewicz and Hamilos, 2010). SP, CGRP and neurokinin A (NKA) (and their respective receptors NK-1, CGRP1 and NK-2) are reportedly involved in the late phase response in allergic nasal obstruction in guinea pigs (Kaise, 2001).
The release of pro-inflammatory cytokines such as TNFα and IL-4 from degranulated mast cells promotes the differentiation of CD4+ T helper cells into Th2 phenotype. This Th2 response promotes the production of eosinophils and the phenotype switching of B lymphocytes leading to increased production of IgE and increased proliferation and activation of mast cells (Deo et al., 2010). The weighting of Th1/Th2 balance towards Th2 characterises the allergic response (Deo et al., 2010).

1.3.1.1 The role of neuropeptides in airway inflammation

Non-opioid pro-inflammatory neuropeptides contribute to neurogenic inflammation by promoting vasodilation and plasma extravasation, notably in the nasal mucosa in allergic rhinitis (Sarin, 2006). The nasal mucosa has dense networks containing the pro-inflammatory neuropeptides SP, VIP and CGRP which can arise from sensory and autonomic nerve fibres and from neuroendocrine cells found widely in the nasal mucosa (Hauser-Kronberger, 1997). SP has also been shown to be co-localised with TRPV1 and the neurotrophin receptor tyrosine kinase trk-A in airway-specific murine dorsal root ganglion neurons (Dinh, 2004). TRPV1 positive cells are found on epithelial cells, vascular endothelial cells, submucosal glands and sensory nerves in human nasal mucosa (Seki et al., 2006). TRPV1 receptor is activated by several triggers including capsaicin, noxious heat (42-53^{o}C), low extracellular pH, ethanol, acids, pollution, protons and lipids (Dinh, 2004; Geppeti, 2006). TRPV1 receptor activation may mediate the production and release of S P (Dinh, 2004). The expression and sensitivity of TRPV1 receptor can be up-regulated by the neurotrophin NGF (Aloe and Manni, 2009). In allergic airway inflammation (including allergic asthma and allergic rhinitis) SP and CGRP levels in the saliva and nasal secretions are elevated (Hauser-Kronberger, 1997;

Nockher, 2006). SP in the nasal mucosa of humans increases eosinophil accumulation during repeated allergen exposure in allergic rhinitis (Fajac et al., 1995).
SP and CGRP both activate monocytes to release the pro-inflammatory cytokines TNFα, IL-1β, IL-6 and IL-10 (Cuesta, 2002; Renz, 2001). In allergic airways inflammation, SP stimulates Th1/Th2 phenotype switching in T cells and immunoglobulin switching in B cells (Nockher, 2005). SP also up-regulates expression of macrophage inflammatory protein 1β (MIP-1β) in human T lymphocytes (in vitro) (Guo et al., 2002).
SP and CGRP often act synergistically and also potentiate each other in inflammatory oedema, in plasma extravasation during airways inflammation and in mast cell degranulation (Brain, 1985; Brokaw, 1992; Reynier-Rebuffel, 1994; Wu, 2006). NGF activation of the high-affinity NGF receptor tyrosine kinase trkA can generate production and release of SP, while SP, in turn, can promote the production and release of NGF (Dinh, 2004; Nockher, 2006; Skoff, 2006). NGF (and to a lesser extent, glial cell-derived neurotrophic factor [GDNF]) can also promote CGRP content and release from TRPV1-expressing trigeminal ganglion neurons in vitro, while BDNF had no effect on CGRP (Price, 2005).
From this evidence it can be seen that pro-inflammatory neuropeptides such as SP, CGRP and VIP interact with various immune cells including T lymphocytes, B lymphocytes, magrophages, monocytes and mast cells to modulate allergic inflammation of the nasal mucosa. These interactions influence the release of cytokines and are capable of modifying Th1/Th2 balance in CD4+ T cell differentiation. Pro-inflammatory neuropeptides can act synergistically and potentiate each other. Pro-inflammatory neuropeptides and neurotrophins can promote each other's production and release. This complex cross-talk between inflammatory cells, cytokines, neuropeptides and neurotrophins during allergic mucosal inflammation and the influence acupuncture may have on these interactions is the central focus of this study.

1.3.1.2 The role of neurotrophins in airway inflammation

Neurotrophins, or nerve growth factors, are proteins which regulate the survival, death or differentiation of neurons. The primary function of neurotrophins is to promote nerve growth. The main categories of neurotrophins include Nerve Growth factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), Glial cell-derived Neurotrophic Factor

(GDNF), Neurotrophin 3 (NT-3) and Neurotrophin 4/5 (NT 4/5). The density of innervation to the nasal mucosa in allergic rhinitis patients is reported to be double that of healthy individuals (Anggard et al., 1983; Figueroa et al., 1998; Hauser-Kronberger, 1997). Much of this additional innervation surrounds arterial blood vessels (Figueroa et al., 1998). In allergic rhinitis this neuronal abundance is likely to contribute to hypersensitivity as well as to amplifying the allergic inflammatory response. Concentrations of NGF, BDNF and NT-3 increase dramatically in the respiratory epithelium during allergic rhinitis (Kemi et al., 2006; Nockher, 2006). Both nasal NGF and BDNF (brain-derived neurotrophic factor) expression were reported to be significantly increased in allergic rhinitis patients compared to healthy controls after nasal allergen provocation (Pfaar et al., 2009). This allergen-induced increase in BDNF correlated with the maximal increase in total nasal symptom score (TNSS), suggesting an important role for this neurotrophin in modifying symptom severity in allergic rhinitis patients (Pfaar et al., 2009).

Monocytes produce, store and release NGF, BDNF and NT-3 and hence are a potential source of elevated neurotrophin levels in the nasal mucosa (Rost, 2005). In addition to promoting neuronal proliferation, neurotrophins also prolong the survival of eosinophils and mast cells, thereby prolonging the inflammatory response (Nockher, 2006). Eosinophils, mast cells and macrophages, in turn, produce NGF, with some researchers reporting that eosinophils are the main source of NGF, while mast cells account for only a small fraction (Nockher, 2006; Wu, 2006). Hence sources of neurotrophins in allergic inflammation of the nasal mucosa include inflammatory cells (monocytes, eosinophils, mast cells and macrophages) and the nasal epithelium. Wu et al reported that the major sources of NGF in the human nasal mucosa are submucosal glands, nasal epithelium and eosinophils (Wu, 2006).

During allergic asthma, airway epithelial cells express increased amounts of the neurotrophins NGF and BDNF, which in turn promotes the survival of tissue eosinophils (Hahn, 2006). This increased epithelial cell production of neurotrophins is up-regulated by interleukin 1 beta (IL-1β), TNFα and Th2 cytokines (Hahn, 2006). Human bronchial smooth muscle can also express NGF, BDNF and NT-3, with IL-1β capable of stimulating expression of NGF and BDNF, but not NT-3 (Kemi et al., 2006). In chronic allergic asthma, neurotrophins in the bronchi and lungs are derived mainly from airway epithelial cells but also from bronchial smooth muscle and inflammatory cells (Hahn, 2006; Kemi et al., 2006). Glaab et al reported that, in allergic asthma, NGF

is directly involved in early-phase airway response (EAR), but has no effect on the inflammatory response or late-phase airway response (LAR) (Glaab, 2003).
As previously mentioned, NGF and SP promote each other's production and release (Dinh, 2004; Nockher, 2006; Skoff, 2006).
Taken together these findings suggest not only an important role for SP, CGRP and VIP in promoting and amplifying allergic airways inflammation, including allergic rhinitis, but also suggest a complex interaction between inflammatory cells, cytokines and neurotrophins with these pro-inflammatory neuropeptides.

1.3.2 The burden of disease: prevalence and cost

Worldwide, allergic rhinitis is estimated to affect 18% of 15-34 year olds and 10% of 35-54 year olds (AccessEconomics, 2007). Studies estimate that allergic rhinitis affects approximately 11.9% to 30.2% of the general population of the United States of America, with an even greater prevalence in children (Bousquet et al., 2008; Nathan et al., 2008; Wallace et al., 2008). Prevalence of allergic rhinitis in Europe is estimated to range from 17% in Italy to 29% in Belgium (Bauchau and Durham, 2005). In China, prevalence rates for allergic rhinitis range from 8.7% to 37.9% with wide variations between regions and higher prevalence in urban populations (Zhang and Zhang, 2014). There is also marked diversity in allergic rhinitis prevalence in Africa (7.2% to 54.1%), Latin America (5.5% to 45.1%) and the Middle East (7.4% to 45.2%) (Katelaris et al., 2012). The overall trend shows an increase in prevalence of allergic rhinitis worldwide, especially in urban populations and more affluent regions (Katelaris et al., 2012; Zhang and Zhang, 2014).

Allergic rhinitis is a significant burden to the Australian community in quality of life and wellbeing, impaired performance, loss of productivity and health care costs. The total number of Australians who have allergic rhinitis is estimated to be in the order of 3.7 million based on self-reported data from the Australian Health Survey (ABS, 2013) . Although a physician-derived estimate of allergic rhinitis national point prevalence in 2000 was 18.7% (Walls et al., 2005), according to the National Health Survey self-reported point prevalence in 2001 was 15.5%. The most recently published Australian Health Survey (2011-2012) shows an increase in self-reported "hayfever and allergic rhinitis" to 16.8% (ABS, 2013) (see Table 1).

Table 1. National point prevalence of self-reported hayfever and allergic rhinitis in Australia according to Australian Bureau of Statistics National Health Surveys

1995	2001	2004-2005	2007-2008	2011-2012
13.9%	15.5%	16.1%	15.1%	16.8%

Other studies suggest that the prevalence of allergic rhinitis continues to increase worldwide (Dykewicz and Hamilos, 2010).

Table 2. National point prevalence of self-reported hayfever and allergic rhinitis in Australia by age group according to Australian Bureau of Statistics Australian Health Survey (2011-2012)

0–14 years	15–24 years	25–34 years	35–44 years	45–54 years	55–64 years	65–74 years	75 years and over
9.5%	18.8%	21.9%	22.5%	18.9%	14.1%	14.3%	12.1%

For Australian 15-24 years old, "hayfever and allergic rhinitis" was the most frequently reported long-term condition in the National Health Surveys in 2004-2005 (19%), 2007-2008 (17%) and 2011-2012 (18.8%) (ABS, 2006, 2009, 2013).

By state, in the ABS National Health Surveys since 2001, the highest prevalence of allergic rhinitis has been consistently reported in ACT, while the lowest was in New South Wales and Queensland. Between 2007-2008 and 2011-2012 surveys, prevalence of allergic rhinitis has increased in all states and territories (ABS, 2002, 2006, 2009, 2013).

Table 3. Point prevalence of self-reported hayfever and allergic rhinitis by state and territory according to Australian Bureau of Statistics National Health Surveys

	ACT	SA	WA	VIC	TAS	NSW	QLD	NT
2001	25.2%	20.05	18.3%	17.5%	16.2%	13.1%	13.2%	-
2004-2005	21.3%	21.0%	18.7%	18.1%	15.0%	13.8%	14.1%	-
2007-2008	20.7%	17.3%	19.6%	17.6%	16.9%	13.1%	11.5%	-
2011-2102	22.1%	19.9%	20.9%	19.0%	19.6%	15.6%	12.4%	12.4%

Abbreviations: ACT – Australian Capital Territory, SA – South Australia, WA – Western Australia, VIC – Victoria, TAS – Tasmania, NSW - New South Wales, QLD – Queensland, NT – Northern Territory

Allergic rhinitis has also been identified as a risk factor in the development of adult-onset asthma, increasing risk by 3-5 times (Guerra, 2002). The clinical connections between allergic rhinitis and asthma have been highlighted by the ARIA group, as previously mentioned (Bousquet et al., 2008; Braunstahl, 2009; Togias, 2003). Apart from the health burden associated with asthma, in 2004, 313 Australian deaths were attributed to asthma as the underlying cause (ABS, 2006). It is interesting to note that clinical connections between the lungs, nose, large intestine and skin were first postulated in the Yellow Emperor's Classic of Internal Medicine [Huang Di Nei Jing] compiled between the 1st and 5th centuries BC (Kaptchuk, 1983).

A recent study in the US estimated at-work productivity losses from allergic rhinitis to range from $2.4 – 4.6 billion (Crystal-Peters et al., 2000). In a study of 8,267 US workers, Lamb et al reported mean total productivity losses due to allergic rhinitis were US$593 per employee per annum (Lamb, 2006). In 2007, the cost of allergies (excluding allergic asthma) to the Australian health system was estimated to be $349.3 million (AccessEconomics, 2007). Allergic asthma cost a further $808.0 million (AccessEconomics, 2007). The cost of all allergies to the Australian community in 2007 has been estimated at $6.6 billion in lost productivity and other non health-related costs (AccessEconomics, 2007). No separate figures for allergic rhinitis are available.

1.3.3 Treatment options for allergic rhinitis

Numerous oral and intranasal medications are available for the treatment of allergic rhinitis including oral and intranasal anti-histamines, decongestants and corticosteroids, intranasal cromolyn sodium, intranasal anticholinergics, oral anti-leukotreine agents, Omalizumab and topical saline (Wallace et al., 2008).

Allergen immunotherapy has been shown to be effective for the treatment of allergic rhinitis and may reduce the risk of future development of asthma (Wallace et al., 2008).

There is no surgical treatment for allergic rhinitis but some co-morbid conditions that contribute to nasal obstruction (such as severe deviation of the nasal septum or inferior turbinate hypertrophy) may benefit from surgery (Wallace et al., 2008).Complementary and Alternative Medicine (CAM) has become an increasingly popular range of treatment choices for sufferers of allergic rhinitis, with therapies including herbal medicines, homeopathy and acupuncture .(Resnick et al., 2008)

1.4 What is acupuncture?

1.4.1 History of acupuncture

Acupuncture is a form of treatment which originated in China around 2,000 years ago. The earliest extant literature to describe acupuncture, The Yellow Emperor's Classic of Internal Medicine, was compiled between one to five centuries BC (Wang, 1987). Acupuncture spread from China to Japan and Korea in the 6th century AD, and subsequently to other parts of Asia. Acupuncture was probably first practised in Australia by Chinese immigrants during the Victorian Gold Rush. The first acupuncture college in Australia was established in 1969 and acupuncture is now taught in several universities and private colleges around Australia. Victoria was the first Australian state to introduce statutory registration of acupuncture practice in 2002, and on 1st July 2012, national registration of acupuncture practice commenced within the framework of the National Registration and Accreditation Scheme (NRAS) implemented by the Chinese Medicine Board of Australia and the Australian Health Practitioners Regulation Agency (AHPRA).

The practice of traditional Chinese acupuncture is supported by a rich historical literary tradition which allows contemporary acupuncturists to access the clinical observations and experiences of many generations of acupuncturists. Acupuncture is also increasingly supported by research with 6,701 controlled trials and 414 systematic reviews of acupuncture listed in the Cochrane Central Register of Controlled Trials and the Cochrane Database of Clinical Reviews in May 2014.

1.4.2 Acupuncture treatment

"Acupuncture" is a word that is believed to have coined by French Jesuit priests in China in the 17th century, based on the Latin words "acus" – needle and "puncture" – to pierce or puncture. The practice of acupuncture is based on a mapped network of acupuncture points which can be activated by several different types of mechanical, thermal and chemical stimulation. The most common form of acupuncture involves penetration of the skin with a very fine filiform needle (typically 0.25mm in diameter, or 32#, much smaller than the finest 26# subcutaneous needles used in Western biomedicine). Acupuncture needles used in Australia are subject to regulation by the Therapeutic Goods Administration, and are generally single-use factory sterilised stainless steel. After insertion, in most traditional Chinese styles of acupuncture, needles are then manipulated using very fine lifting and thrusting actions often combined with very fine rotation. Needles are typically retained for various durations (from a few seconds to hours, depending on the treatment) then removed. During manipulation a sensory response from the patient called "needle sensation" or "deqi" is frequently sought.

Other stimulation methods often used include warming with a burning herb (moxibustion), electrical stimulation with electrodes attached to needle handles (electroacupuncture), massage and point pressing (Chinese tuina massage or shiatsu), low level laser stimulation, the application of herbal pastes over acupuncture points, and point injection (injection of herbs or other substances such as Vitamin B into acupuncture points).

1.4.3 Parameters which influence the effects of acupuncture

Numerous parameters have been identified which can influence the effects of acupuncture, and these parameters represent important variables in both the interpretation and the design of acupuncture research (Birch, 2004):

- ***Treatment dose*** (frequency and duration of treatment sessions) (White, 2008)
- ***Stimulation parameters*** (manual: manipulation, retention time; electro: waveform, frequency, intensity, duration) (Han, 2004a; White, 2008)
- ***Point selection*** – Electroacupuncture at the point LI 10 and LI 11 had different effects on Th2 cytokine levels (Jong, 2006).

- ***Skill and experience of the acupuncturist*** (Birch, 2004; MacPherson, 2010).
- ***Initial state of the organism*** – The anti-inflammatory effects of acupuncture differed significantly between healthy and inflammation model rats (Li et al., 2007a; Sekido et al., 2003).
- ***Species specific effects*** - Different species of mice responded differently both to TNCB inflammatory challenge and electroacupuncture anti-inflammatory effects (Kasahara et al., 1992).
- ***Time of day (circadian variations)*** - Electroacupuncture at different times of day had significantly different effects on left ventricular function in patients with coronary heart disease (Li et al., 1994).

In this study the treatment dose and stimulation parameters have been based on a critical review of previous acupuncture research on allergic rhinitis, with the conclusion to base treatment dose on the study by Xue et al 2007, namely a treatment regimen of twice weekly for 8 weeks (Xue et al., 2007). It was noted in this review that studies which used 1-12 treatment sessions at a frequency of once weekly or less produced less effective results than studies using 12-30 treatments more than once weekly (see Treatment dose section below for details). Stimulation was also similar to that used by Xue et al, namely deeper insertion and stimulating to elicit needle sensation (deqi) in the real acupuncture group but superficial insertion and no stimulation after insertion in the sham group (Xue et al., 2007) (see Intervention section below for details).
Point selection was based on a critical review of previous acupuncture research on allergic rhinitis, as well as contemporary acupuncture texts (see Intervention section below for details).
All acupuncture treatments in this study for both real and sham acupuncture groups (Group A and B) were provided by the candidate who had over 40 years experience as an acupuncturist and held a Bachelor of Health Science (Acupuncture) (Australian College of Natural Medicine) and a Master of Acupuncture with Distinction (University of Western Sydney).
All subjects in this study were 18-45 year olds who suffered from allergic rhinitis (confirmed by allergy testing and clinical examination by an allergy specialist, Professor Peter Smith), so no variability from initial state of the subjects or from species differences would arise.

To limit circadian variability in the effects of acupuncture and circadian variations in the immune function, all treatments in this study were given between 6am and 12md.

1.5 Clinical efficacy of acupuncture for the treatment of allergic rhinitis

A total of 4 systematic reviews have now been published on the clinical efficacy of acupuncture for allergic rhinitis, three of which were published between 2008 and 2009 (Lee et al., 2009; Passalacqua et al., 2006; Roberts et al., 2008; Xiao et al., 2009). Reviewers' conclusions range between "insufficient evidence" to "safe and effective but more high quality RCTs needed".

Evidence of acupuncture efficacy in persistent allergic rhinitis is currently stronger than for intermittent (seasonal) allergic rhinitis (Lee et al., 2009).

In total 26 relevant RCTs were identified, 9 of which were published in English, 12 in Chinese, 4 in German and 1 in Korean. Since the most recent systematic review was written, another pragmatic study involving 5,237 subjects has been published in English which included two randomised arms and one very large non-randomised arm (Brinkhaus et al., 2008). Two large multi-centre RCTs have also been published since the latest systematic review, one on moderate to severe persistent (perennial) allergic rhinitis (PAR) and the other on seasonal (intermittent) allergic rhinitis (SAR) (Brinkhaus et al., 2013; Choi et al., 2013). These studies have strengthened the evidence for the safety and effectiveness of acupuncture for PAR and SAR respectively.

Of these 26 RCTs, 15 studies were available for review: 9 in English, 4 in Chinese, 1 in German and 1 in Korean (see table 4).

Table 4. RCTs of acupuncture for allergic rhinitis available for review

Williamson et al 1996	Rao and Han 2006 [Chinese]
Wolkenstein and Horak 1998 [German]	He et al 2006 [Chinese]
Petti et al 2002	Chen et al 2007 [Chinese]
Xue et al 2002	Li et al 2007 [Chinese]
Magnusson et al 2004	Xue et al 2007
Ng et al 2004	Choi et al 2013
Brinkhaus et al 2004	Brinkhaus et al 2013
Park et al 2005 [Korean]	

1.5.1 The methodological quality of RCTs of acupuncture for allergic rhinitis

The methodological quality of these studies was assessed using the Jadad scale (1996), which allows for a maximum of 5 points. Of the 15 studies, 12 studies scored 3 or more out of 5 which is often used as a threshold for acceptable methodological quality for inclusion in systematic reviews (see Table 5). The use of self-reporting symptom and quality of life questionnaires as the outcome measures made evaluator blinding impossible in the majority of studies (see Table 5).

Table 5. Methodological quality of RCTs of acupuncture for allergic rhinitis using Jadad Scale (1996)

RCTs of acupuncture for allergic rhinitis	1. Described as randomised	2. Adequate randomisation	3. Subject blinding	4. Evaluator blinding	5. Withdrawals and dropouts described	Total Jadad Score (out of 5)
Williamson et al 1996	1	1	1	0	1	**4**
Wolkenstein and Horak 1998	1	1	1	0	0	**3**
Petti et al 2002	1	1	1	0	0	**3**
Xue et al 2002	1	1	1	0	1	**4**
Magnusson et al 2004	1	1	1	0	1	**4**
Ng et al 2004	1	1	1	1	1	**5**
Brinkhaus et al 2004	1	1	1	0	1	**4**
Park et al 2005	1	1	1	1	0	**4**
Rao and Han 2006	1	1	0	0	1	**3**
He et al 2006	1	-1	0	0	0	**0**
Chen et al 2007	1	-1	0	0	0	**0**
Li et al 2007	1	-1	0	0	1	**1**
Xue et al 2007	1	1	1	0	1	**4**
Choi et al 2013	1	1	1	0	1	**4**
Brinkhaus et al 2013	1	1	1	0	1	**4**

Abbreviations: RCT – Randomised controlled trial

1.5.2 The reporting quality of RCTs of acupuncture for allergic rhinitis

When reporting in these 15 RCTs was assessed using the STRICTA (STandards for Reporting Interventions in Clinical Trials of Acupuncture) checklist, treatment regimens (frequency and duration of treatment) and control interventions were adequately described in all studies, while needling details were also well-documented in all but one study (see Table 6) (MacPherson, 2010). Acupuncture rationale was not described in three studies, and co-interventions were poorly described or not reported in 5 studies. The reporting of practitioner background (training and experience of the acupuncturist/s) was very poorly documented in 10 out of the 15 studies (see Table 6). One study stated the acupuncturists were "experienced general practitioner acupuncturists" which falls short of full transparent disclosure (Williamson, 1996). Since the skill level of the acupuncturist is potentially a confounding variable in acupuncture trials, disclosure of the training and clinical experience of the acupuncturist is essential to enable accurate critical analysis (Birch, 2004) .

Table 6. Compliance of RCTs of acupuncture for allergic rhinitis with STRICTA Reporting Guidelines

RCTs of acupuncture for allergic rhinitis	1. Acupuncture rationale	2. Needling details	3. Treatment regimen (number & frequency of treatments)	4. Co-interventions	5. Practitioner background (length of training & clinical experience)	6. Control intervention(s)
Williamson et al 1996	X	/	/	/	X	/
Wolkenstein and Horak 1998	X	X	/	/	X	/
Petti et al 2002	/	/	/	/	X	/
Xue et al 2002	/	/	/	/	X	/
Magnusson et al 2004	/	/	/	/	X	/
Ng et al 2004	/	/	/	/	/	/
Brinkhaus et al 2004	/	/	/	/	/	/
Park et al 2005	/	/	/	X	X	/
Rao and Han 2006	/	/	/	X	X	/
He et al 2006	/	/	/	X	X	/
Chen et al 2007	/	/	/	X	X	/
Li et al 2007	X	/	/	X	X	/
Xue et al 2007	/	/	/	/	/	/
Choi et al 2013	/	/	/	/	/	/
Brinkhaus et al 2103	/	/	/	/	/	/

Abbreviations: RCT – randomised controlled trial , STRICTA – Standards for Reporting Interventions in Clinical Trials
Legend: X –not adequately reported, / - adequately reported

1.5.3 Sample size

Randomised controlled trials of acupuncture for allergic rhinitis have ranged in total sample size from 24 to 150 subjects (see table 7). Five RCTs reported power calculations and these ranged from 26 to 80 subjects per arm to achieve statistical significance. Studies conducted by Xue et al predicted that on the basis of a 70% reduction in nasal symptom scores with real acupuncture and a 30% reduction in sham acupuncture a sample size of 36 for each group was required to provide 80% power with a type 1 error rate of 5% (two-tailed). Using these predictions Xue et al demonstrated significant differences in nasal symptom scores between real and sham acupuncture treatment groups with a type 1 error rate of 1% (Xue et al., 2007). (For details of the power calculation used for this study see Chapter 2 - 2.3.5 Sample size).

Table 7. Sample sizes in randomised controlled trials and one pragmatic study of acupuncture for allergic rhinitis

RCTs of acupuncture for allergic rhinitis	Sample size (total)	Sample size (per arm)	Power calculation (NNT)
Williamson et al 1996	116 (102)	51	58
Wolkenstein and Horak 1998	24	12	
Petti et al 2002	120	30	
Xue et al 2002	30	17+13, 13+17 crossover design	28
Magnusson et al 2004	40	20	
Ng et al 2004	72	35, 37	80
Brinkhaus et al 2004	52	26	26
Park et al 2005	101	50, 51	
Rao and Han 2006	150	50	
He et al 2006	120	60	
Chen et al 2007	135	45	
Li et al 2007	100	50	
Xue et al 2007	80	42, 38	36
Choi et al 2013	238	97,94,47 (2:2:1)	238
Brinkhaus et al 2013	422	212, 102, 108 (2:1:1)	
Pragmatic study	**Sample size (total)**	**Sample size (per arm)**	**Power calculation (NNT)**
Brinkhaus et al 2008	5,237	487, 494 (+4,256 non-randomised)	

Abbreviations: RCTs – randomised controlled trials; NNT- number needed to treat

1.5.4 Treatment dose

Acupuncture treatment dose refers to the frequency and duration of acupuncture treatment regimens in trials. Since the frequency and duration of acupuncture treatment appears to be a significant variable in determining treatment response, the 15 RCTs of acupuncture for allergic rhinitis were analysed on the basis of treatment dose. Studies in which 12 or more treatments were given with a frequency of more than once weekly consistently reported more positive outcomes than those studies giving less than 12 treatments once weekly or less (see Table 8a, 8b). This difference suggests that acupuncture treatment dose may be an important variable for outcomes in acupuncture trials on allergic rhinitis, specifically that inadequate acupuncture treatment dose may lead to suboptimal results (Birch, 2004). In this study the treatment dose selected has been based on the most recent well-constructed study with positive outcomes, namely Xue et al, 2007 in which treatment was given twice weekly for 8 weeks.

Table 8a. Symptomatic improvements in RCTS of acupuncture for AR with treatment frequency of once weekly or less and a total of 1-12 treatments

Authors/Date	Study design	Outcomes
Williamson et al, 1996	***3 or 4 treatments once weekly***	No significant differences between real and sham acupuncture groups in mean weekly symptom scores, mean weekly medication use or perceived effect of acupuncture
Wolkenstein et al, 1998	***9 treatments once weekly*** for 9 weeks	Reduced symptoms in real acupuncture group in 2nd month but "range of scatter too great for statistical significance"
Petti et al, 2002	***1 treatment***	Symptom scores significantly greater decrease in real acupuncture and sham acupuncture group than untreated group For real acupuncture IL-2 and IL-10 significant decrease, IL-6 no significant difference.
Brinkhaus et al, 2004	***6 treatments once weekly*** for 6 weeks	No difference in Allergic Rhinitis Symptom Questionnaire (ARSQ) SF-36 physical - no change; SF-36 mental – significant improvement Significant improvement in real acupuncture/herbs group in symptom scores (VAS), RQLQ and Global Assessment of Change Scale (GACS)
Magnusson et al, 2004	***12 treatments over 4 months***	No significant differences between real and sham acupuncture groups in symptoms (VAS) or laboratory tests (except for significant difference in skin test and specific IgE for one allergen – mugwort)
Park, 2005	***1 treatment***	Statistically significant increase in NV and MCA at 0 min after acupuncture in both active and placebo acupuncture, with greater increase in active acupuncture. At 7.5 min NV and MCA in active acupuncture remained above baseline but increases not significant; NV in placebo group below baseline and MCA insignificantly increased At 15 min NV and MCA in active acupuncture remained above baseline but increases not significant; NV and MCA in placebo group below baseline.

Abbreviations: AR – allergic rhinitis, EA: electroacupuncture, FPS: five-point symptom scale, IgE: Immunoglobulin E, IL-4: Interleukin-4, MCA: Total nasal minimal cross-sectional area (cm^2), NV: nasal volume (cm^3), RQLQ: Rhinoconjunctivitis Quality of Life Questionnaire, SE: standard error, SF-36: 36-item-form heath survey(after Ware et al, 1992, SP: substance P, TNSS: total nasal symptom score, VAS: visual analogue scale, VIP: vasoactive intestinal peptide, vs: versus

Table 8b. Symptomatic improvements in RCTS and pragmatic studies of acupuncture for AR with treatment frequency of more than once weekly and a tota1 of 12 or more treatments

Authors/Date	Study design	Outcomes
Xue et al, 2002	***12 treatments*** ***3 times weekly*** for 4 weeks then crossover	Significant difference in improvement between real and sham acupuncture in favour of real in 5 pt scale symptom score (FPS).
Ng et al, 2004	***16 treatments*** ***twice weekly*** for 8 weeks	Real vs sham acupuncture: significant improvement in daily rhinitis scores, VAS and number of symptom-free days
Rao et al, 2006	***24 treatments*** ***daily*** for 6 days out of 7 for 4 weeks	Symptom scores improved in acupuncture and ear point group significantly more than medication group Serum IgE and IL-4 decreased significantly
He et al, 2006	***30 treatments*** ***Daily*** for 10 days X 3 courses	Asymptomatic – Acupuncture 41 (68.3%), herbs 20 (33.3%) Effective – Acupuncture 10 (16.7%), herbs 16 (26.7%) Ineffective – Acupuncture 9 (15.0%), herbs 24 (40.0%) Total effectiveness rate 85% acupuncture vs 60% for oral herbs ($p<0.01$)
Xue et al, 2007	***16 treatments*** ***twice weekly*** for 8 weeks	8 wks – greater reduction in 7-day TNSS in real than sham; significantly greater reduction in rhinorrhoea in real than sham 12 weeks – greater reduction in 7-day TNSS in real than sham and significantly greater reduction in all 4 nasal symptoms Reduction in relief medication within real acupuncture group at 8 and 12 wks
Li et al, 2007	***30 treatments*** ***Daily*** for 10 days x 3 courses	Significant difference in reduction of symptoms in EA group over medication group. Both groups showed significant lowering of both SP and VIP after treatment vs before treatment Significant difference in lowering of VIP in EA group compared to medication group.
Chen et al, 2007	***20 treatments*** ***Daily*** for 10 days x 2 courses	Significant difference in total effectiveness rate ($p<0.05$) and recurrence rate at six months follow-up ($p<0.05$) Acupuncture 1 - 91.1% , Acupuncture 2 - 71.1%, Herbs - 46.7%.
Brinkhaus et al, 2008	***Up to 15 treatments*** ***over 3 months***	3 months RQLQ score improved by a mean (±SE) of 1.48 (±0.06) in acupuncture group, by 0.50 (±0.06) in control [3 month scores 1.44 (±0.06) and 2.42 (±0.06), difference in improvement 0.98 (±0.08), $p<0.001$]. 6 months improvement less than 3 months
Choi et al, 2013	***12 treatments*** ***3 times weekly*** for 4 weeks	Significant improvement in TNSS after treatment in real acupuncture group compared to sham and waitlist groups Significant improvement in both TNSS and TNNSS compared to baseline in both real and sham acupuncture groups
Brinkhaus et al, 2013	***12 treatments*** ***over 8 weeks***	Significant improvement in RQLQ scores and histamine use compared to sham and medication only groups

Abbreviations: RCTs– randomised controlled trials, AR – allergic rhinitis, VAS – visual analogue scale, IgE – immunoglobulin E, IL-4 – interleukin 4, TNSS – total nasal symptom scores, EA – electroacupuncture, SP – Substance P, VIP – vaso-active intestinal peptide, RQLQ – Rhinoconjunctivitis Quality of Life Questionnaire, SE – standard error, TNNSS – total non-nasal symptom score

1.5.5 Outcome measures and outcomes

1.5.5.1 Subjective outcome measures

The most commonly used outcome measures in randomised controlled trials of acupuncture for allergic rhinitis to date have been symptom and quality of life scores such as:

- Rhinoconjunctivitis Quality of Life Questionnaire (after Juniper et al, 1996) (Brinkhaus et al., 2004; Brinkhaus et al., 2013)
- Allergic Rhinitis Symptom Questionnaire (ARSQ) (Brinkhaus et al., 2004)
- Global Assessment of Change Scale (GACS) (Brinkhaus et al., 2004)
- MOS 36-item-form heath survey (SF-36)(after Ware and Sherbourne, 1992) (Brinkhaus et al., 2004)
- Visual analogue scales of rhinitis symptoms (VAS) (Brinkhaus et al., 2004; Magnusson et al., 2004; Ng et al., 2004; Wolkenstein and Horak, 1998)
- Total Nasal Symptom Scores (TNSS) and Total Non-nasal Symptom Scores (TNNSS) (Choi et al., 2013)
- Six-point scale for rhinorrhoea, nasal itching, sneezing, mouth breathing, nocturnal cough, nasal blockage (Petti et al., 2002)
- Five-point scale (FPS) for sneezing, watery rhinorrhoea, nasal congestion, itching nose, itching eyes and eye watering (Xue et al., 2002)
- Four-point scale for nasal itching, nasal obstruction, rhinorrhoea and sneezing (Ng et al., 2004)
- Three-grade score for sneezing, rhinorrhoea, nasal congestion and nasal itching (Rao and Han, 2006)
- Ten-point scale for symptom severity (Williamson, 1996)
- Other undisclosed symptom scales (He et al., 2006; Li et al., 2007b)

This heterogeneity of outcome measures used complicates the task of systematically comparing study outcomes, and makes meta-analysis difficult.

Real acupuncture has been compared to sham acupuncture in a total of 11 randomised controlled trials, and in 10 of these studies improvement in symptoms was measured using one of the abovementioned symptom scoring methods. In 7 studies real acupuncture produced significantly greater improvement in symptoms than sham acupuncture (Brinkhaus et al., 2004; Brinkhaus et al., 2013; Choi et al., 2013; Ng et al., 2004; Petti et al., 2002; Xue et al., 2007; Xue et al., 2002), while

3 studies failed to find a statistically significant difference (Magnusson et al., 2004; Williamson, 1996; Wolkenstein and Horak, 1998). In 2 studies comparing electroacupuncture to medication or body acupuncture, ear acupuncture and medication, electroacupuncture, body acupuncture and ear acupuncture all produced significantly greater symptomatic improvement than medication (Li et al., 2007b; Rao and Han, 2006). In 2 studies comparing acupuncture to Chinese herbs, acupuncture was significantly more effective than Chinese herbs after treatment, and in one study this was maintained at 6 months follow-up (Chen, 2007; He et al., 2006).

In addition, other measures used included symptom-free days (Ng et al., 2004), days on sick leave due to allergic rhinitis (Magnusson et al., 2004) and relief medication use (Brinkhaus et al., 2013; Ng et al., 2004; Williamson, 1996; Xue et al., 2007; Xue et al., 2002). Real acupuncture was shown to be more effective than sham acupuncture in increasing symptom-free days (Ng et al., 2004), while days on sick leave was apparently documented, but not reported (Magnusson et al., 2004). Two studies found no significant change in the use of self-administered relief medication (Ng et al., 2004; Xue et al., 2002). Another study found no significant difference between real and sham acupuncture groups in relief medication use at either 8 or 12 weeks follow-up, however a reduction in relief medication was reported in the real acupuncture group at 8 and 12 weeks compared to baseline (Xue et al., 2007). The most recent multi-centre study found significant reduction in anti-histamine use following real acupuncture compared with sham acupuncture or relief medication only (Brinkhaus et al., 2013).

1.5.5.2 Objective outcome measures

Objective measures of mechanistic outcomes used in randomised controlled trials of acupuncture for allergic rhinitis include serum IgE, IL-2, IL-4, IL-6, IL-10, interferon gamma (IFNγ), SP, VIP, gene expression of interleukin 1 receptor 1 (IL-1R1), blood eosinophils, nasal eosinophils, nasal volume (cm^3) (NV) and total nasal minimal cross-sectional area (cm^2) (MCA).

Findings on serum IgE were mixed, with no significant difference in post-acupuncture IgE reported by some researchers (Ng et al., 2004), but a significant decrease in IgE was found by others (Rao and Han, 2006). Magnusson et al tested total IgE and specific IgE for 5 allergens and found no significant change after acupuncture except for specific IgE for mugwort which significantly decreased (Magnusson et al., 2004). In a small cohort

study (n=22), Lau et al reported a decrease in serum IgE for 64% of subjects post-acupuncture and in 76% of subjects at 2 months follow-up, but to complete the picture, 31% of subjects showed an increase in IgE post-acupuncture, which persisted in 18% of subjects at 2 months follow-up (Lau et al., 1975b). It is noteworthy that the only study to report an unequivocal reduction in IgE provided daily acupuncture treatments 6 days a week for four weeks (Rao and Han, 2006), while the studies which produced mixed results or showed no change in IgE used much less intense treatment regimens (Lau et al, 1975). (Magnusson et al., 2004; Ng et al., 2004)

Changes in cytokines which would be expected to be associated with an improvement in allergic inflammation include down-regulation in Th2 cytokines such as IL-4, IL-6 and IL-10 and pro-inflammatory cytokines such as IL-1, IL-6 and IL-10 accompanied by an up-regulation in Th1 cytokines such as IL-2 and IFNγ. The data on post-acupuncture modulation of cytokines is very scant to date. Petti et al reported a significant decrease in IL-10 immediately after a single acupuncture treatment but no significant change in IL-6, and an unexpected significant decrease in IL-2 (Petti et al., 2002). Since the effects of a single acupuncture treatment are not likely to accurately predict the effects of a substantial course of acupuncture (eg 12 treatments or more delivered more frequently than once a week), it is difficult to interpret the results of this study. Rao et al reported a significant decrease in IL-4 after 24 acupuncture treatments over four weeks, but no change in IFNγ (Rao and Han, 2006). Shiue et al conducted a cDNA microarray analysis (on peripheral blood of 18 humans with allergic rhinitis) for gene expression and found that expression of interleukin-1 receptor-α (IL1R1) was significantly decreased at 2 hours, 24 hours and 4 weeks after acupuncture (Shiue et al., 2008). The authors suggested that this may indicate a shift in Th1/Th2 balance away from Th2 after acupuncture treatment (Shiue et al., 2008). Zheng et al also reported a significant reduction in IL-4 and granulocyte macrophage colony stimulating factor (GM-CSF) with a significant increase in IFNγ (Zheng, 2010).

Table 9. Changes in Th1 and Th2 cytokines in studies of acupuncture for allergic rhinitis

Author/ year	Measurement method	Th2 cytokines					Th1 cytokines	
		IL-1β	IL-4	IL-6	IL-10	GM-CSF	IFNγ	IL-2
Petti at al, 2002	Peripheral blood plasma			No change	-			-
Rao et al, 2006	Peripheral blood serum		-				No change	
Shiue et al, 2008	RNA from peripheral blood	-						
Zheng et al, 2010	Supernatant from peripheral blood monocytes		-			-	+	

Abbreviations: IL-Iβ – interleukin 1 beta, IL-4 – interleukin 4, IL-10 – interleukin 10, GM-CSF – granulocyte macrophage - colony stimulating factor, IFNg – interferon gamma, IL-2 – interleukin 2

Legend: - significant reduction + significant increase

One study has examined the effects of acupuncture on the pro-inflammatory neuropeptides SP and VIP in humans with allergic rhinitis (Li et al., 2007b). SP and VIP were measured in plasma from venous blood using radioimmunoassay. When one group receiving 30 treatments of electroacupuncture was compared to another group receiving medication (Cetirizine 10mg tds), both groups showed a significant lowering of both SP and VIP after treatment compared to pre-treatment (Li et al., 2007b). The electroacupuncture group had a significantly greater reduction in VIP than the medication group, but there was no significant difference between groups in reduction of SP (Li et al., 2007b).

No significant differences in blood or nasal eosinophils after acupuncture were found in the only randomised controlled trial to date which has measured them (Magnusson et al., 2004). In a non-randomised study comparing acupuncture (n=25) with anti-histamine medication (n=20) Chari et al found no change in blood eosinophils in either group (Chari et al., 1988). However in a cohort study (n=22), Lau et al reported a significant reduction in blood eosinophils, ($p<0.01$ after treatment; $p<0.02$ at 2 month follow-up) and percentage of nasal eosinophils ($p<0.05$ after treatment and at 2 month follow-up) (Lau et al., 1975b).

Acoustic rhinometry after a single acupuncture treatment was used in one study to measure nasal volume (cm^3) (NV) and total nasal minimal cross-sectional area (cm^2) (MCA) (Park, 2005b). A statistically significant increase in NV and MCA was reported at 0 min after acupuncture in both active and placebo acupuncture, with a greater increase in the active acupuncture group. At 7.5 minutes NV and MCA in the active acupuncture remained above baseline, but increases were not significant; at 7.5 minutes NV in the placebo group was below baseline and MCA was insignificantly increased. At 15 minutes NV and MCA in the active acupuncture remained above baseline but increases were not significant; NV andMCA in the placebo group were below baseline. This suggests that a single acupuncture treatment has an immediate but very short-lived effect in decreasing nasal congestion in humans with allergic rhinitis.

1.5.6 Other studies of acupuncture for allergic rhinitis

In addition to these 26 RCTs, 1 large pragmatic study has been published, 1 RCT assessing the cost effectiveness of acupuncture (as opposed to efficacy and safety), 2 comparative studies (see table 10), 8 cohort studies, 1 case series (see Table 11) and 3 case studies (see table 12) involving acupuncture for allergic rhinitis as well as 8 cohort studies using therapies associated with acupuncture such as moxibustion, external herbal application on (or injection into) acupuncture points and laser acupuncture.

Table 10. Comparative studies of acupuncture for allergic rhinitis

Author/Year	Study type	Sample size	Acupuncture	Results
Chari 1988	Acupuncture vs antihistamine	45 (25,20)	LI 4, LU 7, LI 20, Dingchuan (M-BW-1), ST 3	Significant improvement in both groups in nasal obstruction, rhinorrhoea, itching and sneezing at end of treatment and at 3 month follow-up. Nasal ciliary clearance rates increased significantly in both groups (more lasting in acupuncture group). In both groups a significant fall in nasal secretions and circulating serum IgA levels but no significant change in blood eosinophils or nasal sinus x-rays.
Lai 1993	Acupuncture vs desensitizing injections	42 (23,19) randomised	BL 13, 20, 23, GB 20, LI 20 + pts for patterns	Short-term cure 16 (69.6%) vs 4 (21.1%) Markedly effective 4 (17.4%) vs 5(26.3%) Improved 1 (4.3%) vs 8 (42.1%) Ineffective 2 (8.7%) vs 2 (10.5%)

Abbreviations: IgA – immunoglobulin A, vs - versus

In a study comparing acupuncture (n = 25) to antihistamine medication (n = 20), significant improvement was observed in both groups in nasal obstruction, rhinorrhoea, itching and sneezing at end of treatment and at 3 month follow-up (Chari et al., 1988). Nasal ciliary clearance rates increased significantly in both groups (more lasting in acupuncture group). In both groups a significant fall in nasal secretions and circulating serum IgA levels was reported, but there was no significant change in blood eosinophils or nasal sinus x-rays in either group (Chari et al., 1988).

In a comparison of acupuncture to desensitization injection immunotherapy in allergic rhinitis, 16 out of 23 (69.6%) subjects in the acupuncture group were asymptomatic after treatment for 6 months and remained so at 12 months follow-up compared to 4 out of 19 (21.1%) subjects in the desensitization group (Lai, 1993). In the acupuncture group 4 (17.4%) subjects had a reduction in symptom score by 5-10 points, compared to 5 (26.3%) in the desensitization group, while 1 acupuncture (4.3%) subject and 8 (42.1%) desensitization subjects showed reductions in symptom scores of 2-4 points. A reduction in symptom score of less than 2 points was seen in 2 (8.7%) acupuncture subjects and 2 (10.5%) desensitization subjects. The five signs and symptoms which were assessed to create the symptom scores in this study were given as nasal obstruction, sneezing, rhinorrhoea, swelling of nasal conchae and pallor of nasal mucosa, but the scoring method was not specified (Lai, 1993).

A total of 8 cohort studies of acupuncture for allergic rhinitis have been published with between 22 and 97 subjects (a total of 424 subjects) (see Table 9). Seven of these cohort studies reported symptomatic improvements while one study found no change (Czubalski et al., 1977). In addition to symptom scores, rhinoscopy was also used in one study while another measured blood eosinophils, nasal eosinophils and serum IgE (Kudaibergenova and Zhaisakova, 2009; Lau et al., 1975b).

Table 11. Cohort studies and case series of acupuncture for allergic rhinitis

Author/Year	Study type	Sample size	Acupuncture	Results
Lau 1975	Cohort study	22	LU 5, LI 4, LI 11, LI 20, GB 20, ST 36, Yintang (M-HN-3), Tung's 44.01 (1.5 cun above the elbow joint and 0.5 cun anterior to lateral epicondyle of humerus)	Significant decrease in 6 point symptom scale ($p<0.001$ for congestion, discharge, sneezing, itch, headache; $p<0.01$ for cough), blood eosinophils, ($p<0.01$ after treatment; $p<0.02$ at 2 mth followup) % nasal eosinophils ($p<0.05$ after treatment and at 2 mth followup) serum IgE decreased in 64%
Czubalski 1977	Cohort study	23		unchanged
He 1990	Cohort study	60	Laser LI 20, LI 4, ST 36	42 (70%) cured after 12 treatments 8 (13.3%) cured after 24 treatments 10 (16.7%) ineffective after 24 treatments
Sun 1993	Cohort study	97		
Ji 1997	Cohort study	76		
Xia 1997	Cohort study	72	Agger nasi points bilaterally (2-15 treatments)	Significant effect 41 (56.9%) Effective 29 (40.2%) Ineffective 2 (2.7%)
Tan 1999	Cohort study	52	LI 20, Yintang (M-HN-3), LI 4, ST 36, + Wind-Cold: BL 12, GB 20 + SP Xu: BL 13, BL 20 + KI Xu: BL 23, GV 23	41 (78.8%) markedly improved 9 (17.3%) improved 2 (3.9%) ineffective
Kudaibergenova 2009	Cohort study	24	Ear acupuncture plus body acupuncture	18 (75%) marked improvement (self-reported s&s and rhinoscopy)
Zhang 2009	Case series	4	Pattern-based individualized treatment	

Table 12. Case studies of acupuncture for allergic rhinitis

Author/Year	Study type	Sample size	Acupuncture
Ji 1994	Case study	1	
Hu 1999	Case study	1	Pattern-based individualized treatment
Nesbitt 2006	Case study	1	

In a recent pragmatic study, Brinkhaus et al divided 5,237 subjects into two randomised arms and a third non-randomised arm (n = 4,256) (Brinkhaus et al., 2013). One randomised group (n = 487) received acupuncture immediately while the second group (n = 494) received acupuncture after a delay of three months, hence no subject blinding was possible. Improvement in clinical signs and symptoms was self-reported using RQLQ and SF-36. At 3 months the immediate treatment group showed significant improvement on RQLQ and SF-36 scores compared to baseline (mean reduction in RQLQ scores of 48.6% (45.2-52.0) and compared to the control group which had not yet received any acupuncture treatment (greater mean reduction in RQLQ of 31.8% (16.5-37.2); $p < 0.001$). All three groups showed significant improvement on RQLQ scores at 6 months follow-up, with the immediate treatment group showing greater improvement than the delayed treatment group ($p = 0.47$) and the non-randomised group showing greater improvement than the two randomised groups ($p = 0.002$). The authors concluded that when acupuncture is added to routine care for allergic rhinitis that "clinically relevant and persistent benefits" are seen. No serious adverse effects were reported in this study, and 529 minor adverse effects reported by 10.8% of patients included minor bleeding and haematoma (69%), needling pain (9%), local infections (4%), vegetative symptoms (2%) and other (16%).

1.6 Mechanisms by which acupuncture may moderate the clinical symptoms of allergic rhinitis

(See Appendix A)

Anti-inflammatory mechanisms of acupuncture in allergic rhinitis

Recent research is beginning to elucidate some of the mechanisms underpinning acupuncture's anti-inflammatory effects. Multiple physiological pathways appear to mediate the anti-inflammatory effects of acupuncture including the HPA axis, sympathetic pathways (via both sympathetic post-ganglionic neurons and sympathoadrenal medullary axis), descending inhibitory pathways (which are similar to the descending inhibitory pathways associated with acupuncture analgesic actions) and possibly parasympathetic cholinergic pathways.

Other relevant anti-inflammatory effects of acupuncture include an anti-histamine effect and down-regulation of pro-inflammatory cytokines (such as TNF-α, IL-1β, IL-6 and IL-10), and pro-inflammatory neuropeptides (such as SP, CGRP and VIP). The

involvement of both opioid and non-opioid neurotransmitters has been demonstrated. Of the opioid neuropeptides, μ-receptor ligands such as β-endorphin, met-enkephalin and endomorphin-1appear to play a prominent role in suppressing inflammation. Neurotrophins (such as NGF, BDNF and NT-3) which can not only contribute to hypersensitivity, but can also enhance and prolong inflammatory response, have been shown to be down-regulated by acupuncture. Acupuncture has also been found to suppress the expression of cyclooxygenase 1 and 2 (COX-1, COX-2) and inducible nitric oxide synthase (iNOS) during experimentally-induced inflammation. N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid kainate receptor (AMPA/KA) receptors (receptors for glutamate and aspartate) have also been implicated in the anti-inflammatory actions of acupuncture.

1.6.1 HPA (hypothalamus-pituitary-adrenal) Axis

In a carageenan-induced paw inflammation rat model, the anti-oedema effects of electroacupuncture were abolished by various disruptions of the HPA axis including adrenalectomy and antagonizing corticotropin releasing hormone (CRH), adrenocorticotropic hormone (ACTH) or glucocorticoid receptors (Li et al., 2008; Li et al., 2007a). The involvement of the HPA axis in the anti-inflammatory effects of acupuncture was further supported by findings of significant increases in levels of ACTH and corticosterone in the same rat paw inflammation model in response to electroacupuncture (Li et al., 2008; Li et al., 2007a).

In the same studies it was found that disruption of the HPA axis had no effect on the anti-hyperalgesic effects of acupuncture (Li et al., 2008; Li et al., 2007a). Other studies have reported that disruption of the HPA axis (adrenalectomy) does not alter electroacupuncture suppression of leucocyte migration (in a mouse air pouch inflammation model) (Kim et al., 2007). Hypophysectomy (removal of pituitary gland) partially blocked electroacupuncture suppression of delayed type hypersensitivity in 3 different species of mice (Kasahara et al., 1993). This suggests that suppression of hyperalgesia and leucocyte migration are anti-inflammatory effects of acupuncture which are not mediated by the HPA axis, and that acupuncture suppression of delayed type hypersensitivity is only partially mediated by the HPA axis.

1.6.2 Sympathetic pathways

Leucocyte migration appears to be mediated by activation of β-2 adrenoreceptors on leucocytes by noradrenalin released from sympathetic post-ganglionic neurons in

response to low-frequency electroacupuncture (Kavoussi and Ross, 2007; Kim et al., 2007). Low frequency electroacupuncture suppression of carageenan-induced paw oedema in mice also appears to be mediated via sympathetic post-ganglionic neurons (Kim et al., 2008). On the other hand, high frequency electroacupuncture effects in suppressing carageenan-induced paw oedema appear to be mediated via the sympathoadrenal medullary axis (Kim et al., 2008). These conclusions were based on a study using the carageenan hind-paw inflammation model in imprinting control region (ICR) mice which tested four interventions – adrenalectomy, peripheral chemical sympathectomy, a β-adrenoreceptor blocker and a glucocorticoid receptor blocker (Kim et al., 2008). Adrenalectomy had no effect on low frequency (1 Hz) electroacupuncture anti-inflammatory actions, but abolished high frequency (120 Hz) electroacupuncture suppression of paw odema and myeloperoxidase (MPO) activity as well as significantly decreasing electroacupuncture anti-hyperalgesia (Kim et al., 2008). Peripheral chemical sympathectomy with 6-hydroxydopamine (6-OHDA) destroyed sympathetic post-ganglionic neurons while sparing sympathetic innervation to the adrenal medulla, which resulted in a decrease in low frequency electroacupuncture's effects on paw oedema, MPO activity and hyperalgesia, but did not alter these anti-inflammatory effects in high frequency electroacupuncture (Kim et al., 2008). Propranolol, a β-adrenoreceptor blocker abolished all anti-inflammatory effects of both low and high frequency electroacupuncture (Kim et al., 2008). A glucocorticoid receptor blocker, RU-486, had no effect on either low or high frequency electroacupuncture's anti-inflammatory effects (Kim et al., 2008).

1.6.3 Conflicting data

Within the studies of the role of the HPA axis and sympathetic pathways in the anti-inflammatory effects of acupuncture, there are two examples of conflicting data. The first relates to effect of adrenalectomy, which blocked the anti-oedema effects of acupuncture in one study (Li et al., 2007a) but had no effect on paw oedema suppression in another study (Kim et al., 2008).

The second discrepancy arises from the use of glucocorticoid receptor blocker, RU-486, which in one study completely blocked the anti-oedema effects of acupuncture (Li et al., 2007a) but in another study had no effect on acupuncture suppression of oedema (Kim et al., 2008). Whilst there were differences in the species of test animal, the

inflammatory medium and the acupuncture points used between these two studies, the reasons for these difference in outcomes are unclear.

1.6.4 Descending inhibitory pathways

Li et al proposed that the anti-hyperalgesia effects of electroacupuncture might be mediated via both serotonergic pathways from the nucleus raphe magnus (NRM) to the dorsal horn of the spine and noradrenergic pathways from the locus coeruleus to the dorsal horn (Li et al., 2007a). A descending inhibitory serotonergic pathway from nucleus raphe magnus to the dorsal horn of the spine has been previously demonstrated to participate in acupuncture analgesia (Takeshige et al., 1992). Similarly, a noradrenergic descending inhibitory pathway from paragigantocellular nucleus in the medulla oblongata to the spinal dorsal horn has also been identified in acupuncture analgesia, however the involvement of noradrenergic neurons arising from the locus coeruleus in the pons has not been previously shown (Takeshige et al., 1992). In this study the authors do demonstrate the existence of these two proposed pathways, and that lesioning of the dorsolateral funiculus in the spine abolishes the electroacupuncture-induced suppression of hyperalgesia (Li et al., 2007a). However the authors' conclusions that this is due to the interruption of the serotonergic and adrenergic pathways in question is problematic, given that the dorsolateral funiculus also has a high density of both SP and enkephalin containing fibres with the highest density found in rexed laminae I and II (Ai, 1986). SP and enkephalin have both been shown to have potent actions in acupuncture analgesia, so the possibility, even probability, that pathways other than the serotonergic and adrenergic pathways studied would be interrupted by lesioning the dorsolateral funiculus should also be considered.

1.6.5 Parasympathetic cholinergic pathways

A parasympathetic anti-inflammatory pathway mediated by acetylcholine has been demonstrated in research not related to acupuncture. Acetylcholine (ACh) released by the vagus nerve in the organs of the monocyte-macrophage system binds to alpha 7 nicotinic receptors (α7nAChR) on macrophages which inhibits the release of pro-inflammatory cytokines (Borovikova et al., 2000; Kavoussi and Ross, 2007; Pavlov et al., 2003; Tracey, 2002). It has been proposed that this cholinergic anti-inflammatory pathway may be activated by acupuncture (Kavoussi and Ross, 2007) however no direct experimental confirmation of this hypothesis is currently available.

1.6.6 Anti-histamine action

Acupuncture has demonstrated an anti-histamine action in both rabbits and humans (Pfab et al., 2005; Xie and Li, 1985). The spread of Evans Blue dye under the skin of rabbits was used to measure increases in capillary permeability in response to histamine intradermal injection (Xie and Li, 1985). When acupuncture was applied the quantity of Evans Blue leakage as well as the levels of histamine in the dermal tissue were significantly reduced (Xie and Li, 1985). Prophylactic acupuncture (for 15 minutes prior to topical histamine application to the skin) was shown to significantly reduce histamine-induced itch and wheal formation in humans compared with placebo-point acupuncture and no intervention (Pfab et al., 2005).

While an anti-histamine action of acupuncture was reported in both of these studies, no potential mechanisms were investigated.

1.6.7 Cytokines

As already mentioned above, some evidence of a shift in Th1/Th2 balance away from Th2 has been shown in studies of acupuncture treatment of allergic rhinitis in humans, namely a significant reduction in IL10 and IL4 and a significant decrease in gene expression for IL1R1 (Petti et al., 2002; Rao and Han, 2006; Shiue et al., 2008). However these same studies also showed no significant change in IL6 and IFN-γ and an unexpected decrease in IL2 (Petti et al., 2002; Rao and Han, 2006). Studies measuring the effects of acupuncture on Th2 and pro-inflammatory cytokines in other inflammatory conditions have reported significant reductions in IL1β and TNF-α in carageenan-induced hind paw inflammation in rats and expression of IL1β messenger RNA (mRNA) and IL6 messenger RNA in ulcerative colitis rats (Fang et al., 2007; Wu, 1999). Acupuncture has also been found to significantly reduce IL6 and IL10 in humans with asthma and IL6 and TNF-α in collagen-induced arthritic mice (Joos et al., 2000; Yim et al., 2007). Tian et al also reported a significant down-regulation of serum and colonic TNF-α mRNA expression in rats with ulcerative colitis (Tian et al., 2003). Secretion of Th2 promoting cytokines IL4 and IL13 was suppressed after acupuncture in a study using dinitrophenyl-keyhole limpet hemacyanin (DNP-KLH) immunised mice (Park et al., 2004).

Very few studies on the effects of acupuncture on Th1 cytokines have been conducted to date and these few studies have not produced strong evidence to support the notion of an acupuncture-induced shift in Th1/Th2 balance. Liu et al reported a significant

increase in IL2 levels after acupuncture in human outpatients with rheumatoid arthritis (Liu et al., 1993). However, as previously mentioned, a single acupuncture treatment in humans with allergic rhinitis produced a significant reduction in IL2 (Petti et al., 2002). No studies to date have demonstrated an increase in IFNγ in response to acupuncture. Rao et al reported no change in IFNγ in humans with allergic rhinitis, Park reported no change in IFNγ secretion after acupuncture in DNP-KLH immunised mice, while in collagen-induced arthritic mice Yim et al found a significant reduction in IFNγ (Park et al., 2004; Rao and Han, 2006; Yim et al., 2007)

In healthy adults Jong et al reported a significant reduction in IL4 and IL6 and a significant increase in IL2, however these findings should be interpreted with caution since the effects of acupuncture in healthy subjects have been shown to differ significantly from the anti-inflammatory effects of acupuncture in inflammatory conditions (Jong, 2006; Sekido et al., 2003).

To date the evidence to support the assertion that acupuncture may cause a shift in Th1/Th2 helper cell balance, and hence influence allergic inflammation, is suggestive but inconclusive. In the proposed research cytokines which indicate any shift in Th1/Th2 helper cell balance in patients with allergic rhinitis in response to acupuncture treatment will be measured.

1.6.8 Neuropeptides

Studies on the effects of acupuncture on neuropeptides can be divided broadly into research into opioid neuropeptides and non-opioid pro-inflammatory neuropeptides. Much of the research on acupuncture suppression of inflammatory hyperalgesia overlaps with the broader research on acupuncture's anti-nociceptive actions and the role of opioid neuropeptides in these effects. Opioid neuropeptides which have been shown to mediate anti-nociceptive effects of acupuncture include enkephalins, β-endorphin, endomorphins, dynorphins and nociceptin/orphanin FQ and different frequencies of electroacupuncture have been shown to stimulate the production and release of different neuropeptides in a highly selective manner (see Table 13) (Han, 2004b).

Early experiments into the role of opioid neuropeptides in acupuncture-induced anti-noception tended to use a generic opioid-receptor antagonist, Naloxone, however this proved a rather blunt research tool since it was not possible to discriminate which opioid neuropeptides were involved . Naloxone has also been used in a number of

studies of acupuncture's anti-inflammatory effects, including suppression of hyperalgesia, delayed type hypersensitivity, leukocyte migration and inflammatory oedema. Sekido et al found that local Naloxone injection into an inflamed hind paw had much stronger inhibiting effects on acupuncture suppression of hyperalgesia than intraperitoneal Naloxone injection (Sekido et al., 2003). Local injection of Naloxone into an inflamed hind paw as well as intraperitoneal Naloxone were shown to inhibit both manual and electroacupuncture suppression of hyperalgesia (Ceccherelli et al., 2002).
In 2,4,6-trinitrochlorobenzene (TNCB)-induced delayed type hypersensitivity in mice, both intraperitoneal and intracisternal injection of Naloxone completely blocked electroacupuncture suppression of delayed type hypersensitivity (Kasahara et al., 1993). Electroacupuncture suppression of leucocyte migration was shown to be significantly decreased by intraperitoneal injection of Naloxone but unaffected by intrathecal Naloxone injection (Kim et al., 2006).

The effects of low frequency (4Hz) electroacupuncture on reducing carageenan-induced hind paw oedema were not affected by intraperitoneal injection of Naloxone (Zhang et al., 2004b). Other studies have shown that the anti-oedema effects of low frequency electroacupuncture are mainly mediated via the HPA axis and sympathetic post-ganglionic neurons, rather than via central or peripheral opioid pathways, which may account for the anti-oedema effects of acupuncture being unaffected by an opioid antagonist (Kim et al., 2008; Li et al., 2008; Li et al., 2007a).

The actions of specific opioid neuropeptides in the anti-inflammatory effects of acupuncture have been studied by selectively targeting specific opioid receptors.
In a (complete Freund's adjuvant (CFA)-induced hind paw inflammation model in rats, intra-thecal injections of selective antagonists for mu (μ), delta (δ) and kappa (κ) receptors were tested for their effects on acupuncture suppression of hyperalgesia (Zhang et al., 2004a). Electroacupuncture at 10Hz and 100Hz exerted an anti-hyperagesic effect which was blocked by μ and δ receptor antagonists, but κ receptor antagonists had no effect. Selective antagonists for μ and δ receptors would be expected to block the anti-nociceptive actions of the Endomorphins (μ receptor ligands) and Enkephalins (δ receptor ligands) both of which respond to low frequency electroacupuncture (1-4 Hz typically, but 10Hz in this study) (Han, 2004b). However

the suppression of the anti-hyperalgesic effects of 100Hz electroacupuncture by blocking μ and δ receptors (whose ligands are thought to respond only to low frequency electroacupuncture) is unexplained. Similarly dynorphins are κ receptor ligands which respond to 100Hz electroacupuncture, hence κ receptor blocking would be expected to abolish their anti-nociceptive actions, however in this study κ receptor blocking had no impact on 100 Hz electroacupuncture anti-hyperalgesia (Han, 2004b).

In another CFA-induced hind paw inflammation model in rats, Zhang et al used Dermasap, a selective toxin for μ-opioid receptor containing neurons which destroyed 30% of the μ-opioid receptor containing neurons in the lumbar spinal cord while sparing the δ-opioid receptor containing neurons and SP containing neurons (Zhang et al., 2005). This selective destruction of μ-opioid receptor containing neurons completely abolished the anti-hyperalgesic effects of electroacupuncture at 10Hz. The apparently successful antagonism of μ-opioid neuropeptides (such as β-Endorphin and Endomorphins) in this study is predictable, however the question remains why the other unaffected opioid neuropeptides, notably the δ-opioids (such as Enkephalins) failed to exert any residual anti-hyperalgesic action.

Table 13. Opioid neuropeptides and their receptors

Receptors	μ mu	δ delta	κ kappa	ORL1/NOP
Natural ligands	endomorphins (EM-1, EM-2) endorphins (β-EP)	enkephalins (MEK, LEK)	dynorphins (DYN-A, DYN-B)	nociceptin/ orphanin FQ (N/OFQ)
Weak ligands	[enkephalins] (MEK, LEK)	[endorphins] (β-EP)		

Abbreviations: ORL1/NOP – orphanin FQ/nociceptin receptor**,** EM-1 – endomorphin 1, EM-2 – endomorphin 2, MEK – Met-Enkaphalin, LEK – Leu-Enkephalin, DYN-A – dynorphin A, DYN-B – dynorphin B, β-EP – beta endorphin, N/OFQ – nociceptin/orphanin FQ

Acupuncture has been reported to lower plasma levels of SP and VIP in patients with persistent (perennial) allergic rhinitis (Li et al., 2007b). This finding supports the hypothesis first presented in this proposal that acupuncture may down-regulate SP, VIP and CGRP in humans with allergic rhinitis. Decreased levels of SP and VIP were also closely correlated with improvements in clinical signs and symptoms (Li et al., 2007b) (see Figure 1).

Both irritable bowel syndrome (IBS) and allergic rhinitis involve inflammatory responses in mucosal immunity, so it is feasible that the actions of acupuncture on IBS may have some relevance to the actions of acupuncture in allergic rhinitis. In rats with IBS, acupuncture has been shown to prevent activation of mast cells in the intestinal mucosa and to down-regulate secretion of SP and VIP (Wu, 2008). Another recent study reported that acupuncture can decrease the number of mast cells and down-regulate the expression of SP and SP receptor (SPR/NK-1R) in the colonic mucosa of IBS rats as well as down-regulate the expression of Corticotropin Releasing Hormone (CRH) in the hypothalamus (Ma, 2009).

In patients with migraine, acupuncture has been shown to lower plasma levels of SP and CGRP (Li, 2001). Low frequency electroacupuncture (10Hz) has been found to reduce Neurokinin-1 receptor (NK-1R), the receptor for which SP is the natural ligand, in the spinal dorsal horn. The opioid neuropeptide Enkephalin inhibits or regulates SP release from peripheral nerve endings via activation of opiate receptors, suggesting a possible role for Enkephalin in the down-regulation of SP by acupuncture (Ai, 1986; Kondo, 2005; Yonehara et al., 1992).

1.6.9 Neurotrophins

Acupuncture has been reported to both up-regulate and down-regulate neurotrophins, particularly NGF and brain-derived neurotrophic factor (BDNF), depending on the disease and animal model being studied (Bai et al., 2004; Liang et al., 2002; Manni et al., 2005; Pagani et al., 2006; Stener-Victorin et al., 2000; Stener-Victorin, 2003; Wang et al., 2002). In a rodent model of Parkinson's disease acupuncture has been reported to increase the production of endogenous BDNF in subtantia nigra cells (Liang et al., 2002). In retinitis pigmentosa, NGF levels in tears increased after acupuncture (Pagani et al., 2006). In steroid-induced polycystic ovarian syndrome in rats, acupuncture has been found to decrease NGF in the ovaries (Bai et al., 2004; Stener-Victorin et al., 2000) and to prevent up-regulation of the generic neurotrophin receptor p75 (Manni et al., 2005). In experimental spinal cord injury in cats and rats, acupuncture increased the expression of NT-3 (Wang et al., 2002; Yan et al., 2009) as well as BDNF, NGF, GDNF and FGF (fibroblast growth factor) (Chen et al., 2007; Wang et al., 2007; Wang et al., 2005).

To date no human studies have measured the effects of acupuncture on modulation of neurotrophins in allergic rhinitis. In this study, the effect of acupuncture treatment on NGF and BDNF levels in mucosal secretions in patients with allergic rhinitis is examined.

1.6.10 Other mediators and receptors

In carageenan-induced inflammation in rats, acupuncture has been reported to reduce levels of prostaglandin E_2 (PGE_2), COX-1, COX-2 and iNOS, while alleviating inflammatory oedema and hyperalgesia (Lee, 2006). COX-2 and iNOS expression were also significantly inhibited, along with pro-inflammatory cytokines TNFα, IL-1β and IL-6 in a rat spinal cord injury model (Choi et al., 2010).

In carageenan-induced hind paw inflammation rat model, electroacupuncture and NMDA or AMPA/KA receptor antagonists have been shown to exert a synergetic anti-nociceptive action against inflammatory pain, an action which appears to be due to an acupuncture-induced reduction of glutamate and aspartate (which bind to NMDA and AMPA/KA receptors) in the spinal dorsal horn (Zhang et al., 2002).

1.7 The research question

Given the complex cross-talk between cytokines, neuropeptides and neurotrophins in allergic inflammation, and in the light of various actions of acupuncture on cytokines, neuropeptides and neurotrophins which have been reported in a variety of other contexts, it was hypothesised that acupuncture might exert anti-inflammatory actions in humans with allergic rhinitis.

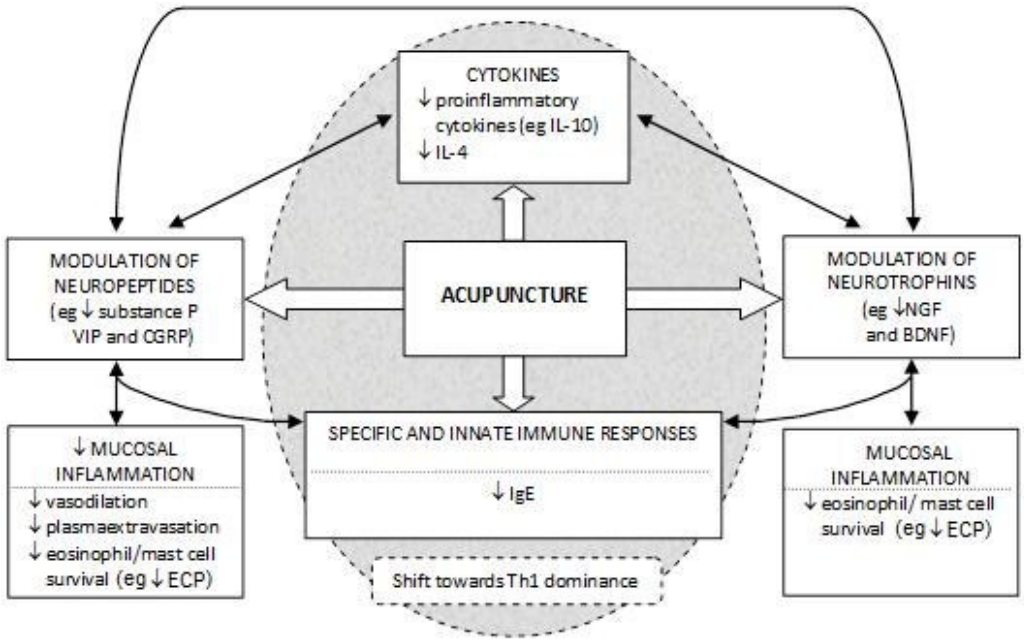
The purpose of this study was to examine any potential modulation of mucosal immunity in the nasal airway following acupuncture treatment for persistent allergic rhinitis (see Figure 1). It was hypothesised that any potential modulation might be associated with changes in neuropeptides, neurotrophins, cytokines and immunoglobulins. The selection of the biomarkers measured in the study was based on the review of the anti-inflammatory effects of acupuncture seen in animals and humans in previous studies which is detailed in Appendix A.

According to the proposed model, a down-regulation of the pro-inflammatory neuropeptides SP, CGRP and VIP after acupuncture would be accompanied by a reduction in mucosal inflammation. To measure any reduction in mucosal inflammation, nasal airways were monitored and symptoms and quality of life assessed.

Potential down-regulation of pro-inflammatory cytokines and Th2 cytokines (and possible up-regulation of Th1 cytokines) after acupuncture would be associated with a shift towards Th1 dominance and a reduction in IgE.

Potential down-regulation of neurotrophins NGF and BDNF, in response to acupuncture, would also be associated with a reduction in mucosal inflammation and eosinophil and mast cell survival (see Figure 1). Hence, in addition to measurement of nasal airway resistance, symptoms and quality of life, ECP was measured to monitor eosinophil survival.

Figure 1. Proposed model for the mechanism of acupuncture in mucosal inflammation



Abbreviations: BDNF – brain-derived neurotrophic factor; CGRP – calcitonin gene-related peptide; IgA – Immunoglobulin A; IgE – Immunoglobulin E; IgG – Immunoglobulin G, IgM – Immunoglobulin M; IL-4-Interleukin 4; IL-10- Interleukin 10; NGF; NK cell-natural killer cell; VIP-vaso-active intestinal peptide, Th1 – Th1 phenotype of T helper cells
NB: Other immunoglobulins, lysozyme, lactoferrin and NK cell activity, which were originally included in the research proposal, could not be measured due to limitations of the study. Potential modulation of TRPV1 receptor by acupuncture was also discussed in the published review (see Appendix A) but was not measured in this study.

Chapter 2: Materials and Methods

2.1 Introduction

Between October 2009 and May 2012, 151 volunteers with diagnostically-confirmed persistent (perennial) allergic rhinitis (PAR) were recruited to a three arm subject-and-assessor-blinded randomised sham-controlled trial of acupuncture treatment for PAR. Although the practice persists of describing randomised controlled acupuncture trials as "double blind" where the subject and assessor are both blinded but the acupuncturist is not (Balk, 2009; Hasegawa, 2013), the term "subject-and-assessor-blinded" avoids any ambiguity about whether or not the acupuncturist is blinded (Choi et al., 2013; Park, 2005a). The study was conducted in Southport on the Gold Coast in Queensland, Australia between two centres, a private allergy clinic (Queensland Allergy Services) and a private acupuncture clinic (Southport Acupuncture Clinic). Laboratory testing was carried out in the laboratories of Griffith University's Gold Coast campus, and also at Queensland Medical Laboratories (QML), a commercial pathology service.

The study was funded by the National Health and Medical Research Council (grant 536564), approved by the Griffith University Human Research Ethics Committee (GU Ref No: MED/02/09/HREC), and registered with the Australian New Zealand Clinical Trials Registry (ACTRN 12610001052022).

After the treatment phase of the study was completed in August 2012, a further group of non-atopic healthy volunteers was recruited and tested for the same parameters as the allergic participants (except for symptom questionnaires, as they were asymptomatic), to provide "normal range" data for these parameters.

Outcome measurements included clinical assessments, biomarkers and self-administered questionnaires and a daily symptom and medication diary. Clinical assessments included an inspection of the inferior turbinates by rhinoscopy to estimate obstruction caused by swelling, and measurement of peak nasal inspiratory flow (PNIF). Biomarkers were measured in blood serum, blood plasma and saliva, and nasal fluids were also collected from the non-atopic group.

Previous studies of the acupuncture treatment of persistent allergic rhinitis have tended to use either exclusively subjective or exclusively objective outcome measurements. In

this study, the simultaneous measurement of both subjective and objective outcome measurements was undertaken with the goal of exploring any correlations between subjective and objective outcomes.

2.2 Acupuncture study design

The study was a three arm subject-and-assessor-blinded randomised sham-controlled trial. In this study the acupuncturist was not blinded to group allocations, however subjects in the real acupuncture and sham acupuncture groups were blinded to group allocation, and this blinding was later confirmed to have been successful by the exit debrief questionnaire results (see section 4.5.3). Clearly the subjects in the no acupuncture group were aware of their group allocation. Staff at the allergy clinic who carried out clinical assessments were blinded to subjects' group allocations. Laboratory staff who performed tests on saliva and blood were blinded to group allocations. The candidate was blinded to group allocations during data entry and statistical analysis by recoding of the groups by a Griffith University staff member who was not involved in the study.

Initial screening and recruitment was conducted at the allergy clinic in Week 0 (see Table 1). After testing and recruitment at the allergy clinic, peripheral venous blood samples were collected at QML situated in the same building as the allergy clinic. Acupuncture treatment commenced the following week at the acupuncture clinic, and was given twice weekly for 8 weeks (Week 1 to 8). Saliva samples were collected at the acupuncture clinic by the candidate in Weeks 1, 3 and 6. An exit debrief questionnaire was completed by subjects in the real and sham acupuncture groups after the final treatment in Week 8 at the acupuncture clinic. In Week 9 and Week 12 subjects returned to the allergy clinic for follow-up testing. In Week 12 daily symptom and medication diaries were handed in at the allergy clinic and a final blood sample was collected at QML.

Table 1. Study design chart

Week 0	Week 1	Week 2	Week 3	Week 4 and 5	Week 6	Week 7 and 8	Week 9	Week 10 and 11	Week 12
Allergy clinic	Acupuncture clinic						Allergy clinic		Allergy clinic
screening, recruitment randomisation	2 acupuncture treatments per week								
SPT, PNIF, rhinoscopic examination, iTNSS, MiniRQLQ							PNIF, rhinoscopic examination, iTNSS, MiniRQLQ		PNIF, rhinoscopic examination, iTNSS, MiniRQLQ
Saliva samples: SP, VIP, ECP	Saliva samples: SP, VIP		Saliva samples: SP, VIP		Saliva samples: SP, VIP	Exit debrief	Saliva samples: SP, VIP		Saliva samples: SP, VIP, ECP
	Daily symptom and medication dairy								
QML									QML
blood samples: RAST, IgE, CGRP, NGF, BDNF, IL-2, IL-4, IL-10, IL-12(p70), IFNγ, eotaxin									blood samples: RAST, IgE, CGRP, NGF, BDNF, IL-2, IL-4, IL-10, IL-12(p70), IFNγ, eotaxin

Abbreviations: SPT – skin prick test, PNIF – peak nasal inspiratory flow, iTNSS – instantaneous total nasal symptom scores, exit debrief – exit debrief questionnaire, MiniRQLQ – Mini Rhinoconjunctivitis Quality of Life Questionnaire, SP – substance P, VIP – vaso-active intestinal peptide, ECP – eosinophilic cationic protein, QML – Queensland Medical Laboratories, RAST – radio-allergoabsorbent test, IgE – immunoglobulin E, CGRP – calcitonin gene-related peptide, NGF – nerve growth factor, BDNF – brain-derived neurotrophic factor, IL-2 – interleukin 2, IL-4 – interleukin 4, IL-10 – interleukin 10, IL-12(p70) - interleukin 12(p70), IFNγ – interferon gamma,

2.3 POPULATION

Study participants were adults aged from 18 to 45 years (mean age 28.0±7.5 years) who suffered from persistent allergic rhinitis (n = 151). Another small group of 18 to 45 year-old non-atopic healthy adults (n = 20) was also recruited to provide baseline "normal range" values for measurements used in the study (see Chapter 6).

Selection of subjects for the acupuncture study

2.3.1 Recruitment and randomisation

Intake of volunteers for the trial began in October 2009 and was continuous until May 2012. A randomisation schedule was generated before recruiting commenced, using the Research Randomizer tool (http://www.randomizer.org/ accessed in September 2009). An additional 20 lines were added to the schedule in April 2012 again using Research Randomizer to replace places lost to exclusions and withdrawals. Only the research assistant who entered the subjects' names into the randomisation schedule at

recruitment and the candidate who provided the acupuncture treatments had access to the randomisation schedule.

Methods of advertising for subjects included broadcast emails to Griffith University students and staff and media releases which generated newspaper and magazine articles, television news coverage and a story on a national television current affairs program. Small posters were displayed and handbills were distributed at a gymnasium, a TAFE college, a college of natural health and various pharmacies. An advertisement was also placed in a local newspaper.

All enquirers were provided with information about the study including the inclusion and exclusion criteria for the study, and details of the location and timing of study participation. Those enquirers who believed they were eligible for inclusion in the study, and who were interested in participating, were then telephoned by the candidate to confirm eligibility and availability to attend the allergy clinic and acupuncture clinic. Once this was confirmed, contact details were forwarded to the allergy clinic to arrange initial appointments. After each subject was assessed at the allergy Clinic, confirmed to be eligible and signed the consent form, the research assistant entered the subject in the randomisation schedule on the next vacant line (which thereby allocated the subject to Group A (real acupuncture), B (sham acupuncture) or C (no acupuncture)) and contacted the subject to arrange their appointments at the acupuncture clinic. The candidate was also sent an email advising the ID and group allocation of new subjects.

2.3.2 Inclusion Criteria

The inclusion criteria for the study were: Males and females aged 18 – 45 years; more than a 2-year history of moderate to severe allergic rhinitis; positive skin prick test to three clinically relevant local grass pollens (Bahia grass, Johnson grass and Bermuda grass) and/or house dust mite (*Dermatophagoides pteronyssinus*); radio-allergosorbent test to house dust mite or Bermuda grass.

2.3.3 Exclusion Criteria

The exclusion criteria for the study were: Non-allergic rhinitis; negative on skin prick test to three local grass pollens and/or house dust mite and negative blood test to house dust mite or Bermuda grass; acupuncture treatment for allergic rhinitis or specific immunotherapy in the previous 2 years; respiratory diseases e.g. asthma, nasal

polyposis and Chronic Obstructive Pulmonary Diseases; infectious diseases; medications that affect salivary flow and components e.g. systemic corticosteroids, diuretics and some antidepressants (during the initial assessment, the allergologist will decide whether the subject is eligible for the study); pregnancy; nasal recreational drugs.

2.3.4 Standardised medication regime

Subjects who participated in the study were permitted to use a topical nasal steroid spray (eg: Mometasone furoate, Budesonide or Fluticasone propionate) and a non-sedating antihistamine (eg: Cetirizine hydrochloride, Fexofenadine hydrochloride or Loratadine) prescribed by the allergologist for symptomatic relief medication. Use of symptom medication was discouraged in the 48 hours prior to assessment. The use of any medications was recorded on a daily diary chart. Subjects were advised to continue prescribed medications for other conditions.

2.3.5 Sample size

A power analysis was performed using Sample Power™. Based on previous human clinical studies and human and animal studies immunological responses it was estimated that a sample size of 25 in each group was required to ensure an 80% power with a type 1 error rate of 5%. Previous studies conducted by Xue et al (Xue et al., 2007; Xue et al., 2002) predicted that on the basis of a 70% reduction in nasal symptom scores with real acupuncture and a 30% reduction in sham acupuncture a sample size of 36 for each group was required to provide 80% power with a type 1 error rate of 5% (two-tailed). Using these predictions Xue et al (Xue et al., 2007) demonstrated significant differences in nasal symptom scores between real and sham acupuncture treatment groups with a type 1 error rate of 1%. Hence, for this study we chose to recruit 36 subjects to each group. This would allow for a 10% dropout rate in each group and still retain sufficient power to detect statistical differences between the study groups. Although symptom change was a secondary outcome in this study (the primary outcome being laboratory evidence of modulation of mucosal immunity), the Xue et al study was the best available evidence at that time for estimating sample size.

2.4 INTERVENTION

Real Acupuncture Treatment

2.4.1 Acupuncture point selection

The rationale for acupuncture point selection in this study was based on three sources of data – points frequently used in research studies, points frequently recommended in modern acupuncture textbooks in the clinical sections, and finally points for which indications relevant to allergic rhinitis frequently appear in the point indications sections of modern texts (Beijing College of Traditional Chinese Medicine, 1980; Brinkhaus et al., 2004; Chamfrault, 1964; Cheng, 1987; Choe and Lee, 1973; Chuang, 1972; Ellis et al., 1988; Magnusson et al., 2004; Ng et al., 2004; O'Connor and Bensky, 1981; Omura, 1982; Petti et al., 2002; Wood and Lewith, 1998; Wu et al., 1999; Xue et al., 2007; Xue et al., 2002).

From an analysis of published acupuncture studies, the most commonly used acupuncture points for allergic rhinitis have been: LI 20 (Yingxiang), LI 4 (Hegu), Yintang (M-HN-3) and ST 36 (Zusanli). Of these points, both ST 36 and LI 4 have been shown to influence immunomodulation (Liu et al., 2009b; Petti et al., 1998; Zhang et al., 2011).

Other points which have been used by at least two researchers include GB 20 (Fengchi), LU 7 (Lieque) and LR 3 (Taichong) (see Table 2).

Modern acupuncture texts, in clinical sections, recommend the following points for rhinitis: Yintang, LI 20, LI 4, ST 36, GB 20, LU 7, LR 3, GV 23 (Shangxing), Bitong (M-HN-14) and BL 10 (Tianzhu) (see Table 2).

Modern acupuncture texts, in point indications sections, list rhinitis or indications related to rhinitis (such as rhinorrhoea and sneezing) for the following points: Yintang, LI 20, LI 4, GB 20, GV 23 and Bitong. For ST 36, LU 7 and LR 3 no relevant indications were found in the searched texts (see Table 2).

The following points were chosen for this study: Yintang, LI 20, LI 4, ST 36 and GV 23. This selection of points incorporated the most commonly used points in research studies to date, including ST 36 and LI 4 for which research also supports a specific role in immunity. An additional point, GV 23, was included due to the strong support for

this point in the reviewed acupuncture texts for treatment of allergic rhinitis. Points which were not easily accessible to needle with the subject lying in a supine position were excluded (GB 20 and BL 10), in order to make the treatment both convenient and comfortable for the subject. All points were located according to the WHO International Standard Acupuncture Point Locations in the Western Pacific Region (WHO, 2008). A recent multi-centre study in China and South Korea used exactly the same points as were used in this study with the addition of one other point, ST 3 (Face-Juliao) (Choi et al., 2013).

Table 2. Comparative table of acupuncture points for allergic rhinitis

Acupuncture point	Research literature	Texts – Clinical Section	Texts – Point indications
Yintang (M-HN-3)	Wood 1998, Petti 2002, Xue 2002, Brinkhaus 2004, Magnusson 2004, Ng 2004	Shanghai College of TCM 1981, Chuang 1972, Cheng 1987	Beijing College of TCM 1980, Shanghai College of TCM 1981, Chuang 1972, Ellis 1988, Cheng 1987
LI 20 (Yingxiang)	Wood 1998, Petti 2002, Xue 2002, Brinkhaus 2004, Magnusson 2004	Shanghai College of TCM 1981, Chuang 1972, Cheng 1987	Beijing College of TCM 1980, Shanghai College of TCM 1981, Choe 1973, Chuang 1972, Chamfrault 1964, Ellis 1988, Cheng 1987, Omura 1982
LI 4 (Hegu)	Wood 1998, Petti 2002, Magnusson 2004	Shanghai College of TCM 1981, Chuang 1972, Cheng 1987	Cheng 1987
ST 36 (Zusanli)	Petti 2002, Magnusson 2004, Ng 2004	Chuang 1972	-
GB 20 (Fengchi)	Xue 2002, Brinkhaus 2004	Shanghai College of TCM 1981, Chuang 1972	Beijing College of TCM 1980, Shanghai College of TCM 1981, Ellis 1988, Cheng 1987, Omura 1982
LU 7 (Lieque)	Brinkhaus 2004, Magnusson 2004	Cheng 1987	-
LR 3 (Taichong)	Brinkhaus 2004, Magnusson 2004	Chuang 1972	-
GV 23 (Shangxing)	-	Shanghai College of TCM 1981, Chuang 1972	Beijing College of TCM 1980, Shanghai College of TCM 1981, Choe 1973, Chuang 1972, Chamfrault 1964, Ellis 1988, Cheng 1987
Bitong (M-HN-14)	-	Shanghai College of TCM 1981, Cheng 1987	Shanghai College of TCM 1981, Cheng 1987
BL 10 (Tianzhu)	-	Shanghai College of TCM 1981	Beijing College of TCM 1980, Shanghai College of TCM 1981, Chuang 1972, Chamfrault 1964, Ellis 1988, Cheng 1987, Omura 1982

Shaded points were those chosen for this study

2.4.2 Duration and frequency of acupuncture treatment

A review of previous studies on acupuncture for allergic rhinitis indicated that a treatment regime of twice weekly treatments for 6 to 8 weeks produced significantly superior clinical outcomes when compared to studies which used shorter or less frequent treatments (Brinkhaus et al., 2004; Chuang, 1972; Kim and Park, 1998; Lau et al., 1975a; Magnusson et al., 2004; Ng et al., 2004; Petti et al., 2002; Rao and Han, 2006; Wood and Lewith, 1998; Xue et al., 2007; Xue et al., 2002). Therefore, on the basis of the available evidence, in this study twice weekly treatments for 8 weeks were adopted, with a 2-4 day break between treatments.

2.4.3 Needle technique

Traditional Chinese style single-use disposable stainless steel needles (0.25 x 40mm) (CandG® Acupuncture Needles, Helio Supply Co Pty Ltd, Sydney) were used. The needles were inserted at the indicated points but not manipulated. The needles were retained for 20 minutes (without manipulation) and then removed.

2.4.4 Time of treatment

All acupuncture treatments were performed between 6.00am and midday, twice weekly. This was to limit the influence of circadian variations in mucosal immunity and also circadian variations in the effects of acupuncture as potentially confounding variables in the study.

2.5 COMPARISON

Sham Acupuncture Treatment

2.5.1 Acupuncture point selection

For sham acupuncture a total of 8 needling sites were used to match the number of needling sites in the verum acupuncture group. In the sham group four sites (needled bilaterally) were selected which did not correspond to the locations of known acupuncture points and did not lie on identified acupuncture channels (meridians). To mirror the real acupuncture points used, the sham sites included four needles on the head/face (two bilateral points), two needles on the dorsum of the hand (one bilateral

point) and two needles on the leg (one bilateral point). Midline points on the head were avoided as this would lie on an identified acupuncture channel, the Governor Vessel (Dumai). The point location method was described in the same traditional terms as the real acupuncture points: Forehead sham point: 1 cun lateral to GB 14 (Yangbai); Cheek sham point: The midpoint of the line between LI 20 and SI 19 (Tinggong); Hand sham point: On the dorsum of the hand, at the midpoint of the second metacarpal bone on the ulnar side of the bone; Leg sham point: On the lateral aspect of the lower leg, 1 cun below the point Linghou (M-LE-24).

Non-channel points used in sham acupuncture protocols have been shown not to be inert (Liu et al., 2009a; Wu et al., 2002), however since there is no sham acupuncture protocol which has been validated as inert (Birch, 2006; Dincer and Linde, 2003), needling non-channel points was the most appropriate invasive sham protocol available.

2.5.2 Duration and frequency of acupuncture treatment

The frequency and duration of sham acupuncture treatment were matched to the same frequency and duration as the real acupuncture group, namely twice weekly for 8 weeks.

2.5.3 Needle technique

The same type of needles were used in the sham acupuncture protocol as in the verum acupuncture protocol, namely traditional Chinese style single-use disposable stainless steel needles (0.25 x 40mm) (CandG® Acupuncture Needles, Helio Supply Co Pty Ltd, Sydney). The needles were inserted superficially at the indicated sham sites but not manipulated. The needles were retained for 20 minutes (without manipulation) and then removed.

2.5.4 Time of treatment

All acupuncture treatments were performed between 6.00am and midday, twice weekly. This was to limit the influence of circadian variations in mucosal immunity and also circadian variations in the effects of acupuncture as potentially confounding variables in the study.

2.6 OUTCOMES

2.6.1 Primary and secondary outcomes

2.6.1.1 Primary Outcome

Modulation of the mucosal immune response in the upper respiratory tract by acupuncture in subjects with allergic rhinitis.

2.6.1.2 Secondary Outcome

A reduction in markers of mucosal inflammation (nasal airway resistance, symptoms and ECP) and improved quality of life scores following acupuncture.

2.6.2 Clinical Assessment

A clinical assessment of all participants was conducted by the allergologist at week 0, 9 and 12. At week 0 each participant was assessed to ensure they met the study inclusion criteria. Further clinical assessments on weeks 9 and 12 were conducted to determine if there was any clinical improvement in the week immediately following the period of treatment and whether or not any improvement was still evident one month later.
The clinical assessment included a rhinoscopic examination, by the allergologist, to assess occlusion of nasal airways at the level of the inferior turbinates in each nostril. The clinical assessment was complemented by peak nasal inspiratory flow measurement (PNIF), a daily symptom and medication diary and miniRQLQ, as well as laboratory measures, and were recorded in a case report form. Both the allergologist and the clinical assistant at the allergy clinic were blinded to acupuncture treatment group allocation.

2.6.2.1 Skin prick test (SPT)

SPTs were conducted according to standard protocol (Walls, 1997). Participants were skin prick tested for house dust mite, Bermuda grass, Johnson grass and Bahia grass by the clinical assistant at the allergy clinic at week 0 to establish eligibility for inclusion in the trial (or in the case of the non-atopic healthy controls, exclusion from the trial).

2.6.2.2 Peak nasal inspiratory flow (PNIF)

PNIF was measured by the clinical assistant at the allergy clinic using anterior active rhinomanometry according to the International Committee for the Standardization of Rhinomanometry (ICSR) guidelines (Clement, 1984). The PNIF meter consists of an anaesthesia mask attached to a Youlten peak flow meter which measures nasal airflow in litres per minute. In preparation for the PNIF test the patient was asked to blow his/her nose. After the patient exhaled maximally, the anaesthesia mask was placed over the mouth and nose to achieve an airtight seal. The patient was then requested to inhale forcefully through the nose with the mouth closed. The best of three attempts with less than 10% variation was recorded in litres per minute (Scadding et al., 2011). PNIF was completed at week 0, 9 and 12.

2.6.2.3 Mean inferior turbinate obstruction

Rhinoscopic examination was also performed by the allergologist to estimate obstruction due to swelling at the level of the inferior turbinates expressed as a percentage. The estimates of obstruction from left and right nostrils were then averaged to provide a mean inferior turbinate obstruction score (as a percentage) at weeks 0, 9 and 12.

2.6.2.4 Instantaneous Total Nasal Symptom Score (iTNSS)

Participants were asked to rate four nasal symptoms (runny nose, itchy nose, stuffy nose and sneezing) in severity from 0 (not at all) to 3 (severe) during each visit to the allergy clinic in weeks 0, 9 and 12. (see Appendix C for the complete questionnaire).

2.6.2.5 Mini Rhinoconjunctivitis Quality of Life Questionnaire (MiniRQLQ)

Participants completed a previously validated quality of life questionnaire which was specifically designed for assessment of allergic rhinitis, the Mini Rhinoconjunctivitis Quality of Life Questionnaire (MiniRQLQ) (Juniper et al., 2000). The MiniRQLQ consists of 14 questions across 5 domains. It was completed at week 0, 9 and 12 at the allergy clinic (see Appendix D for the complete questionnaire).

2.6.2.6 Daily Symptom and Medication Diary

Over the study period all participants were requested to complete a daily diary to record seven symptoms (nasal itch, eye itch, sneezing, runny nose, post nasal drip, unrefreshed sleep and sinus pain) and record medications taken (see Appendix E for the complete diary format).

2.6.2.7 Exit debrief questionnaire

Participants in the real acupuncture group (Group A) and the sham acupuncture group (Group B) completed an exit debrief questionnaire at the acupuncture clinic immediately after the last acupuncture treatment in week 8. Participants placed the completed questionnaire in a sealed envelope and returned it to the candidate who then wrote "A" or "B" on the back of the sealed envelope and stored the envelopes in a locked filing cabinet until the completion of data entry. This was to preserve the blinding of the candidate to responses to the questionnaire until data entry was completed.

The exit debrief questionnaire consisted of two questions:

Q1. Do you believe that the acupuncture treatment you received in this study was real acupuncture or sham acupuncture?

Q2. Are there any reasons why you have formed this belief?

2.6.3 Laboratory Assessment

2.6.3.1 Saliva Sample

The real acupuncture and sham acupuncture groups had a saliva sample collected at weeks 0, 9 and 12 at the allergy clinic, and immediately prior to and 4-6 hours and 18-24 hours after each acupuncture treatment at weeks 1, 3 and 6. The no acupuncture group had a saliva sample collected in weeks 0, 9 and 12 at the allergy clinic, and at the acupuncture clinic at weeks 1, 3 and 6. Saliva samples from the non-atopic healthy control group were collected at a single visit to the allergy clinic.

2.6.3.2 Blood Sample

Each atopic participant had a blood sample taken at week 0 and 12. Non-atopic healthy controls gave a blood sample immediately after completing other testing at the allergy clinic.

2.6.3.3 Nasal fluid sample (healthy control group only)

Nasal fluid was collected only from 20 non-atopic healthy adults in order to compare levels of SP, VIP and ECP in saliva versus nasal fluids, and to compare levels of NGF and CGRP in plasma versus nasal fluids.

2.6.4 Sample Collection and Handling

2.6.4.1 Rationale for using saliva

Human studies of mucosal immunity have always been complicated by design difficulties, ethical considerations and subject compliance. It is now well established that saliva, a mucosal secretion that is relatively non-invasive to collect and can be collected in sufficient volume for multiple assays, provides a representative sample of mucosal immune status, particularly of the upper respiratory tract including the nasal passages (see review) (Gleeson et al., 1995).

2.6.4.2 Saliva collection

Samples of mixed saliva were collected using cotton cellulose eyespears as described by our group previously (Strazdins et al., 2005). This collection procedure normally took around 10 minutes. Saliva was collected by the clinical assistant at the allergy clinic and by the candidate at the acupuncture clinic. The time taken to collect each sample was recorded and the volume collected calculated by weight so that approximate salivary flow rates could be determined and the secretion rate of analytes estimated. Following collection, the samples were placed immediately in a -18^{0}C freezer and transported to the laboratory where the samples were frozen in liquid nitrogen and stored at –70^{o}C until further processed. When the sample was thawed it was centrifuged at 4^{oC} at 1000g to recover the saliva and used for analysis or refrozen in 0.5ml aliquots for further analysis. Neat samples of saliva and plasma were used for all assays, except for BDNF, which used a 1/20 dilution of plasma.

2.6.4.3 Blood collection

For blood collection subjects were referred to a pathology collection facility (QML Southport) and a 12.5 ml blood sample was collected by venupuncture into a plain and heparinised tube. Serum samples were frozen at –70^{o}C in 0.5ml aliquots until required for analysis.

2.6.4.4 Nasal fluid collection

The nasal fluid collection was performed by applying two sprays of saline spray to each nostril, followed by an incubation time of 5 minutes. With the subject's head in a vertical position, a nasal tampon was then placed into each nostril for 10 minutes. The nasal tampons were supplied by Ivalon Surgical Products from New London, Conneticut, USA, were described as "post-op sinus pack, K9, with string" and measured 3.5cm x 0.9cm x 1.2cm. The nasal tampons were taken out of each nostril and placed into a 50ml tube, which was immediately placed into a -20°C freezer.
To extract the nasal fluid out of the nasal tampons they were defrosted for 30 minutes and each tampon was soaked in 0.75ml Dulbecco's phosphate buffered saline for 10 minutes on ice. The tampons were then put in a syringe, which was placed on a sterile pipette tip to avoid contact between the syringe and the extracted liquid after spinning. The syringe containing the nasal tampons and the pipette tip were returned to the original 50ml tube and spun for 10 minutes at 2000g and 4°C. From the extracted nasal fluid 4 aliquots were made and stored at -80°C.

2.6.5 Laboratory Analysis

Laboratory analysis was performed with the assistance of Griffith University laboratory staff who were blinded to subjects' group allocations, except for specific antibodies which were tested by a commercial pathology service, whose staff were also blinded to subjects' group allocations.

2.6.5.1 Cytokines

Cytokines indicative of a Th1 response (IFN-γ, IL12), a Th2 response (IL4) and pro-inflammatory cytokines (IL-2, IL-10 and eotaxin) in neat plasma were assessed by means of enzyme-linked immunosorbent assay (ELISA) kits following the

manufacturer's instructions. The kits used were EMD Millipore Human Cytokine/Chemokine Magnetic Bead Panel HCYTOMAG-60K-06.

2.6.5.2 Specific antibodies

In the allergic rhinitis cohort, specific IgE antibodies to house dust mite, Bermuda grass, Johnson grass (*Sorghum halepense*) and Bahia grass (*Paspalum notatum*) were measured in blood serum by ImmunoCAP (Phadia, USA). All immunoglobulin testing was carried out by QML, a commercial pathology service.

2.6.5.3 Neuropeptides and neurotrophins

Levels of SP and VIP in saliva were assessed by means of enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions. The kits used were: SP –HNP-35K-01 supplied by EMD Millipore, St Charles, Missouri, USA; VIP –E90380Hu supplied by USCN Life Science Inc, Wuhan, Hubei, People's Republic of China.

Calcitonin gene-related peptide (CGRP), nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) were measured in plasma using enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions (after levels in saliva were found to be below minimum detection threshold). Kits used were: CGRP –E90876Hu supplied by USCN Life Science Inc, Wuhan, Hubei, People's Republic of China; NGF – Human Adipokine kit HADK2-61K-BO1 supplied by EMD Millipore, St Charles, Missouri, USA; BDNF – Chemikine BDNF Sandwich ELISA CYT306 supplied by EMD Millipore, St Charles, Missouri, USA. All assays were performed with neat saliva or plasma except for the BDNF assay which used a 1/20 dilution.

2.6.5.4 Eosinophilic cationic protein (ECP)

ECP was measured in neat saliva by an enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's instructions. The kit used was Mesacup 7618E supplied by Medical and Biological Laboratories International, Woburn, Massechusetts, USA .

2.6.6 Statistical analysis

Data entry and statistical analysis were performed by the candidate. As the candidate was not blinded to subjects' group allocations, a Griffith University staff member who was not a member of the research team recoded the groups. Hence during data entry and analysis, the candidate was blinded to subjects' new group allocations.

All values from laboratory analysis were standardised across plates (using mean ratios of all available duplicate tests) prior to statistical analysis. For each analyte measured in this study, the Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine normality. For any analyte that did meet the criteria for normal distribution, corresponding parametric statistical methods such as Paired samples T tests were used. For any analyte that did not meet the criteria for normal distribution, data was log-transformed and then re-tested with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Paired samples T tests were then used with log-transformed data whenever the log-transformed data was found to be normally distributed. The Friedman Test was used to determine significant differences over time for each analyte which was not normally distributed after log transformation. Between-group analysis was conducted using Independent Samples T tests for normally distributed data and Independent-samples Kruskal-Wallis tests for non-parametric data. Correlation analyses were conducted using Spearman's rho. Statistical calculations were conducted using SPSS for Windows Version 21. Missing values were excluded from all analyses.

Multivariate analysis of variance (MANOVA) tests were attempted, however since normality tests and multivariate tests failed for most of the data, the underlying assumptions for a valid MANOVA could not be met.

2.6.7 Analytical Strategy

(i) The profile of cytokine production was examined to possibly elucidate the predominant T helper cell type and its importance in the co-ordination of allergic airway inflammation.
(ii) Specific IgE antibodies were measured to determine if, through modulation of the Th1/Th2 balance, acupuncture is able to down-regulate a specific IgE response.

(iii) Acupuncture is known to modulate the release of a number of neuropeptides and neurotrophins, such as SP, CGRP,VIP, NGF and BDNF, all having powerful immunomodulating activities. ECP was measured to determine if there was any effect on eosinophil survival through modulation of neurotrophin by acupuncture.
(v) Acupuncture has been reported to provide symptomatic relief of nasal congestion. Peak inspiratory nasal flow (PNIF) was measured to determine the degree of nasal congestion.
(vi) MiniRQLQs and daily symptom and medication diaries were conducted to assess changes in symptoms and to explore any correlations between symptomatic changes and changes in laboratory measures.
(vii) Data from the real acupuncture group was analysed to determine what percentage of subjects treated showed improvements in signs and symptoms and quality of life.

2.7 Research team

The NHMRC grant team was headed by Prof Allan W Cripps, Pro Vice-Chancellor (Health) from Griffith University (Chief Investigator A – CIA). Professor Peter K Smith, an allergologist, also from Griffith University, was Chief Investigator B (CIB) and Associate Professor Caroline A Smith, an acupuncture researcher from University of Western Sydney was Chief Investigator C (CIC). John L McDonald, an acupuncturist, PhD candidate and Adjunct Senior-Lecturer from Griffith University was Associate Investigator A (AIA) and Associate Professor Brenda Golianu from Stanford University was Associate Investigator B (AIB). Professor Charlie Changli Xue, Head of Health Sciences from RMIT University, was an external PhD supervisor for the candidate. Prof Allan Cripps was the principal PhD supervisor and Professor Peter K Smith was the PhD co-supervisor. Laboratory work was undertaken with the assistance of Dr Ibtisam Ghazawi, Dr Pauline Low and Ann-Christen Bischoff in the laboratories at Griffith University. All acupuncture treatments were provided by the candidate at a private acupuncture clinic. All clinical examinations and tests were performed by Professor Peter K Smith and his clinical assistants at a private allergy clinic.

2.8 Funding

Funding for this study was provided by the National Health and Medical Research Council (NHMRC) grant 536564 (2009-2011) Griffith University - Acupuncture and immunity ($367,650).

2.9 Ethical clearance

Final ethical clearance was obtained from the Griffith University Human Research Ethics Committee on 16th June 2009 for this study entitled "Acupuncture and mucosal immunity in the upper respiratory tract" (GU Ref No: MED/02/09/HREC). An amended clearance was obtained to make changes to saliva collection procedures and changes to blood samples (from 3x5ml samples to 2x12.5ml samples) on 11th October 2009. A further amended clearance was obtained on 4th February 2010 to make changes to the recruiting age range from 18-30 years to 18-45 years. On 29th March 2011 another amended approval was received to add a non-atopic control group and to obtain nasal fluid samples.

2.10 Trial registration

The study was registered with the Australian New Zealand Clinical Trials Registry (ANZCTR) “Accupuncture and mucosal immunity in the upper respiratory tract” (ACTRN 12610001052022). The misspelling of “acupuncture” in the title was corrected in a review submitted in December 2013.

Chapter 3. Baseline demographic, clinical and immunological characteristics of adults with allergic rhinitis: comparisons between the three groups

3.1 Abstract

Background: Baseline demographic, clinical and immunological characteristics of participants randomised to all three groups in the study (real acupuncture, sham acupuncture and no acupuncture) were examined to detect any potential selection bias.

Methods: Parameters of interest included gender, age and symptom severity on recruitment. Clinical measures included an examination to estimate airway obstruction due to swelling at the level of the inferior turbinates, and peak nasal inspiratory flow (PNIF). Laboratory measures (using peripheral blood serum and plasma as well as saliva) included: total and allergen-specific IgE; the neurotrophins NGF and BDNF; the pro-inflammatory neuropeptides, SP, CGRP and VIP; an array of cytokines indicative of Th1/Th2 helper cell balance, and the inflammatory marker, ECP.

Results: There were no statistically significant differences between groups at baseline in gender, age, symptom severity, mean inferior turbinate obstruction, peak nasal inspiratory flow, total IgE, allergen-specific IgE, neurotrophins, pro-inflammatory neuropeptides and ECP. As for the cytokines, there were no significant differences between groups in levels of eotaxin. However there were some significant between-group differences in IL-2, IL-4, IL-10, IL-12 (p70) and IFNγ, with the no acupuncture group showing significantly higher levels of these cytokines than the sham acupuncture group.

Conclusions: Overall, the three randomised groups were well-matched with respect to the parameters measured, except for the levels of cytokines.

3.2 Methods

Baselines measurements for each of the three groups included gender, age and symptom severity on recruitment. Clinical measures included a rhinoscopic examination to estimate airway obstruction due to swelling at the level of the inferior turbinates and

PNIF. Laboratory measures (using peripheral blood serum and plasma as well as saliva) included: total and specific IgE; the neurotrophins NGF and BDNF; the pro-inflammatory neuropeptides, SP, CGRP and VIP; an array of cytokines indicative of Th1/Th2 helper cell balance, and the inflammatory marker, ECP. (For details see Chapter 2).

3.3 Results

3.3.1 Demographics

3.3.1.1 Gender

There was a strongly female gender bias in the numbers of subjects screened (111/152 or 73.0% female) and the number of subjects who completed the trial (81/110 or 73.6% female). This does not reflect the gender balance of adults with allergic rhinitis in the Australian community. The 2011-2012 Australian Bureau of Statistics Australian Health Survey estimated the ratio of females to males with allergic rhinitis in Australia (all age groups included) was 1,918,600:1,783,200 (51.8% female) (ABS, 2013). There were no significant differences at baseline between the real acupuncture, sham acupuncture or no acupuncture groups with respect to gender balance using the Independent-samples Kruskall-Wallis test ($p = 0.073$) (see table 1).

Table 1. Gender ratios across the three groups at baseline

No acupuncture (n = 50)		Sham acupuncture (n = 52)		Real acupuncture (n = 49)	
M:F ratio	% F	M:F ratio	% F	M:F ratio	% F
19:31	62.0%	13:39	75.0%	9:40	81.6%

Abbreviations: M:F ratio – male to female ratio , %F – percentage of females

3.3.1.2 Age

The age range specified in the inclusion criteria for the study was from 18 to 45 years at recruitment date. There were no significant differences at baseline between the real acupuncture, sham acupuncture and no acupuncture groups with respect to age using the Independent-samples Kruskall-Wallis test (p = 0.403) (see table 2).

Table 2. Age distribution across the three groups at baseline

	n	Mean ± SE	Minimum	Maximum
No acupuncture	50	27.7±1.0	18	45
Sham acupuncture	52	29.1±1.1	18	45
Real acupuncture	49	27.2±1.0	19	44

Abbreviations: n – number of subjects, SE – standard error

3.3.1.3 Symptom severity and quality of life scores

There were no significant differences between the real acupuncture, sham acupuncture and no acupuncture groups with respect to symptom severity at baseline [as measured by the Instantaneous Total Nasal Symptom score (iTNSS) (see Tables 3 and 4) or the Mini Rhinoconjunctivitis Quality of Life Questionnaire (MiniRQLQ)] (see Tables 5 and 6). As values for for iTNSS and MiniRQLQ at baseline were not normally distributed on Kolgomorov-Smirnov and Shapiro-Wilk tests for normality, Independent-Samples T tests were performed with logged data (see Tables 4 and 6).

Table 3. Instantaneous total nasal symptom scores (iTNSS) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
49	4.78±0.43	50	5.10±0.37	48	5.25±0.44

Abbreviations: n – number of subjects, SE – standard error, SD – standard deviation

Table 4. Instantaneous Total Nasal Symptom score (iTNSS): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0.07 (-0.05 to 0.19)	0.251	0.01 (-0.09 to 0.11)	0.854	0.06 (-0.05 to 0.17)	0.291

Abbreviations: 95% CI – 95% confidence interval

Table 5. Mini Rhinoconjunctitivitis Quality of Life Questionnaire (MiniRQLQ) scores at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
37	43.62±2.58	37	46.89±2.11	36	44.33±2.72

Abbreviations: n – number of subjects, SE – standard error, SD – standard deviation

Table 6. Mini-Rhinoconjunctivitis Quality of Life Questionnaire (MiniRQLQ): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
-0.01 (-0.11 to 0.09)	0.877	-0.05 (-0.14 to 0.04)	0.258	0.04 (-0.03 to 0.12)	0.234

Abbreviations: 95% CI – 95% confidence interval

3.3.2 Clinical examination results

3.3.2.1 Peak nasal inspiratory flow (PNIF): comparisons between groups

As values for PNIF at baseline were not normally distributed on Kolgomorov-Smirnov and Shapiro-Wilk tests for normality, Independent-Samples T tests were performed with logged data (see Tables 7 and 8).

Table 7. Peak nasal inspiratory flow (PNIF) (Litres/minute) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
49	85.71±5.91	50	82.40±7.52	47	72.87±4.74

Abbreviations: n – number of subjects, SE –standard error, SD – standard deviation

Table 8. Peak nasal inspiratory flow (PNIF) (Litres/minute): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
-0.02 (-0.11 to 0.07)	0.614	-0.03 (-0.14 to 0.08)	0.557	-0.01 (-0.11 to 0.10)	0.876

Abbreviations: 95% CI – 95% confidence interval

3.3.2.2 Mean inferior turbinate obstruction: comparisons between groups

As values for mean inferior turbinate obstruction at baseline were not normally distributed on Kolgomorov-Smirnov and Shapiro-Wilk tests for normality, Independent-Samples T tests were performed with logged data (see Tables 9 and 10).

Table 9. Mean inferior turbinate obstruction (%) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
49	67.96±2.58	50	65.70±2.49	47	69.47±2.25

Abbreviations: n – number of subjects, SE – standard error, SD – standard deviation

Table 10. Mean inferior turbinate obstruction (%): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0.02 (-0.03 to 0.07)	0.482	0.02 (-0.01 to 0.08)	0.169	-0.02 (-0.07 to 0.04)	0.576

Abbreviations: 95% CI – 95% confidence interval

3.3.3 Laboratory results

3.3.3.1 Total and allergen-specific immunoglobulin E (IgE) in serum

As values for serum total IgE and allergen-specific IgE for three local grass pollens and house dust mite at baseline were not normally distributed on Kolgomorov-Smirnov and Shapiro-Wilk tests for normality (but were normally distributed after logging), Independent-Samples T tests were performed with logged data.

3.3.3.1 Total IgE and allergen-specific IgE: comparisons between groups

No significant differences were seen between groups in total IgE (see Table 11 and 12), or in allergen-specific IgE for three local grass pollens (see Tables 13 to 18) or house dust mite (see Tables 19 and 20).

Table 11. Total IgE in serum (kU/L) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
36	329.22±89.25	36	295.56±64.15	34	226.35±72.85

Abbreviations: n – number of subjects, SE – standard error, SD – standard deviation

Table 12. Total IgE in serum (kU/L): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
-0.12 (-0.37 to 0.12)	0.309	-0.11 (-0.36 to 0.14)	0.379	-0.01 (-0.26 to 0.24)	0.916

Abbreviations: IgE – immunoglobulin E, kU/L – kilounits per Litre, 95% CI – 95% confidence interval

Table 13. Specific IgE for Bahia grass in serum (kU/L) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
36	6.97±2.86	36	7.53±3.19	34	9.58±3.27

Abbreviations: n – number of subjects, SE – standard error, SD – standard deviation

Table 14. Specific IgE for Bahia grass in serum (kU/L): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0.16 (-0.21 to 0.54)	0.386	0.24 (-0.14 to 0.62)	0.216	-0.07 (-0.43 to 0.28)	0.678

Abbreviations: IgE – immunoglobulin E, kU/L – kilounits per Litre, 95% CI – 95% confidence interval

Table 15. Specific IgE for Johnson grass in serum (kU/L) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
36	3.55±1.37	36	3.72±1.47	34	4.80±1.66

Abbreviations: n – number of subjects, SE – standard error, SD – standard deviation

Table 16. Specific IgE for Johnson grass in serum (kU/L): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0.13 (-0.18 to 0.45)	0.397	0.18 (-0.14 to 0.50)	0.259	-0.05 (-0.34 to 0.25)	0.748

Abbreviations: IgE – immunoglobulin E, kU/L – kilounits per Litre, 95% CI – 95% confidence interval

Table 17. Specific IgE for Bermuda grass in serum (kU/L) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
36	4.88±2.14	36	5.05±2.49	34	6.85±2.38

Abbreviations: n – number of subjects, SE – standard error, SD – standard deviation

Table 18. Specific IgE for Bermuda grass in serum (kU/L): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0.16 (-0.18 to 0.50)	0.357	0.22 (-0.13 to 0.56)	0.211	-0.06 (-0.37 to 0.25)	0.707

Abbreviations: IgE – immunoglobulin E, kU/L – kilounits per Litre, 95% CI – 95% confidence interval

Table 19. Specific IgE for house dust mite in serum (kU/L) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
36	28.82±5.56	36	30.36±5.43	34	22.76±4.87

Abbreviations: n – number of subjects, SE – standard error, SD – standard deviation

Table 20. Specific IgE for house dust mite in serum (kU/L): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
-0.17 (-0.53 to 0.20)	0.373	-0.19 (-0.57 to 0.19)	0.311	0.03 (-0.33 to 0.39)	0.877

Abbreviations: IgE – immunoglobulin E, kU/L – kilounits per Litre, 95% CI – 95% confidence interval

3.3.3.2 Neurotrophins

As serum values for nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) at baseline were not normally distributed on Kolgomorov-Smirnov and Shapiro-Wilk tests for normality (but were normally distributed after logging), Independent-Samples T tests were performed with logged data.

3.3.3.2 Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF): comparisons between groups

No significant differences were seen between groups in NGF (see Table 21 and 22) or BDNF (see Table 23 and 24).

Table 21. NGF in plasma (pg/ml) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
32	5.13±0.92	32	3.14±0.55	33	3.34±0.59

Abbreviations: NGF – nerve growth factor, pg/ml – picograms per millilitre, n – number of subjects, SE –standard error, SD – standard deviation

Table 22. NGF in plasma (pg/ml): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
-0.15 (-0.37 to 0.07)	0.190	0.04 (-0.18 to 0.26)	0.708	-0.19 (-0.41 to 0.03)	0.094

Abbreviations: NGF – nerve growth factor, pg/ml – picograms per milliitre, 95% CI – 95% confidence interval

Table 23. BDNF in plasma (pg/ml) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
32	2252.06±409.08	32	2023.43±227.97	33	1801.48±146.00

Abbreviations: BDNF – brain-derived neurotrophic factor , pg/ml – picograms per milliitre, n – number of subjects, SE – standard error, SD – standard deviation

Table 24. BDNF in plasma (pg/ml): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0.04 (-0.11 to 0.18)	0.631	0.02 (-0.12 to 0.16)	0.769	0.12 (-0.14 to 0.17)	0.848

Abbreviations: BDNF – brain-derived neurotrophic factor , pg/ml – picograms per milliitre, 95% CI – 95% confidence interval

3.3.3.3 Pro-inflammatory neuropeptides

As salivary values for SP and VIP at baseline were not normally distributed on Kolgomorov-Smirnov and Shapiro-Wilk tests for normality (but were normally distributed after logging), Independent-Samples T tests were performed with logged data.

3.3.3.3.1 Substance P (SP) and vaso-active intestinal peptide (VIP): comparisons between groups

No significant differences were seen between groups at Week 1 (pre-treatment) in salivary levels of SP (see Tables 25 and 26) or VIP (see Tables 27 and 28).

Table 25. SP in saliva (pg/ml) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
32	424.22±119.89	32	455.81±336.74	33	436.92±326.23

Abbreviations: SP- substance P, pg/ml – picograms per millilitre, n – number of subjects, SE – standard error, SD – standard deviation

Table 26. SP in saliva (pg/ml): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
-0.27 (-0.67 to 0.13)	0.188	0.08 (-0.31 to 0.48)	0.680	-0.35 (-0.75 to 0.05)	0.084

Abbreviations: SP- substance P, pg/ml – picograms per millilitre, 95% CI – 95% confidence interval

Table 27. VIP in saliva (pg/ml) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
32	55.02±27.55	32	46.19±14.75	33	50.66±26.00

Abbreviations: VIP- vaso-active intestinal peptide, pg/ml – picograms per millilitre, n – number of subjects, SE – standard error, SD – standard deviation

Table 28. VIP in saliva (pg/ml): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
-0.02 (-0.33 to 0.28)	0.873	-0.11 (-0.42 to 0.19)	0.455	0.09 (-0.21 to 0.40)	0.556

Abbreviations: VIP- vaso-active intestinal peptide, pg/ml – picograms per millilitre, 95% CI – 95% confidence interval

3.3.3.3.2 Calcitonin gene-related peptide (CGRP): comparisons between groups

As plasma levels for CGRP at baseline were not normally distributed on Kolgomorov-Smirnov and Shapiro-Wilk tests for normality, (but were normally distributed after logging), Independent-Samples T tests were performed with logged data . No statistically significant differences were seen between groups in plasma levels of CGRP at Week 0 (p = 0.990) (see Tables 29 and 30).

Table 29. CGRP in plasma (pg/ml) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
32	30.60±4.36	32	29.27±4.50	33	32.78±6.28

Abbreviations: CGRP- calcitonin gene-related peptide, pg/ml – picograms per millilitre, n – number of subjects, SE – standard error, SD – standard deviation

Table 30. CGRP in plasma (pg/ml): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0.02 (-0.19 to 0.23)	0.843	0.04 (-0.18 to 0.25)	0.734	-0.02 (-0.23 to 0.20)	0.884

Abbreviations: CGRP – calcitonin gene-related peptide, pg/ml – picograms per millilitre, 95% CI – 95% confidence interval

3.3.3.4 Other inflammatory markers

3.3.3.4 Eosinophilic cationic protein (ECP): comparisons between groups

As salivary values for ECP at baseline were not normally distributed on Kolgomorov-Smirnov and Shapiro-Wilk tests for normality, even after logging data, Independent-samples Kruskall-Wallis test were performed. No statistically significant differences were seen between groups in salivary levels of ECP at Week 0 (p = 0.816) (see Tables 31 and 32).

Table 31. ECP in saliva (ng/ml) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
35	2.52±0.54	35	3.97±1.08	31	4.06±1.55

Abbreviations: ECP- eosinophilic cationic protein, ng/ml – nanograms per millilitre, n – number of subjects, SE – standard error, SD – standard deviation

Table 32. ECP in saliva (ng/ml): comparisons between groups at baseline

P value (2-tailed)	n	Test statistic	Degrees of freedom
0.816	100	0.407	2

Abbreviations: ECP – eosinophilic cationic protein, nanograms per millitre, n – number of subjects

3.3.3.5 Cytokines

Plasma levels for cytokines at baseline were not normally distributed on Kolgomorov-Smirnov and Shapiro-Wilk tests for normality. Even logged data were only normal for IFNγ and eotaxin. Hence groups were compared using non-parametric analysis with the Independent-samples Kruskall-Wallis test for all cytokines except for IFNγ and eotaxin which were compared using Independent-samples T test with logged data.

3.3.3.5.1 Interleukin 2 (IL-2): comparisons between groups

Independent-samples Kruskall-Wallis test showed significant differences between groups (p = 0.019*) (see Tables 33 and 34). Plasma IL-2 levels at baseline were higher in the no acupuncture group than in both the sham and the real acupuncture groups.

Table 33. IL-2 in plasma (pg/ml) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
32	15.73±10.71	30	3.39±1.88	33	6.06±3.73

Abbreviations: IL-2 – interleukin 2, pg/ml – picograms per millilitre, n – number of subjects, SE – standard error, SD – standard deviation

Table 34. IL- 2 in plasma (pg/ml): comparisons between groups at baseline

P value (2-tailed)	n	Test statistic	Degrees of freedom
0.019*	99	7.940	2

Abbreviations: IL- 2 – Interleukin 2, pg/ml – picograms per millilitre, n – number of subjects

3.3.3.5.2 Interleukin 4 (IL-4): comparisons between groups

Independent-samples Kruskall-Wallis test showed significant differences between groups (p = 0.018*) (see Tables 35 and 36). Plasma IL-4 levels at baseline were higher in the no acupuncture group than in the sham acupuncture group.

Table 35. IL-4 in plasma (pg/ml) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
33	33.31±9.93	32	11.29±5.93	30	32.44±15.12

Abbreviations: IL-4 – interleukin 4, pg/ml – picograms per millilitre, n – number of subjects, SE –standard error, SD – standard deviation

Table 36. IL- 4 in plasma (pg/ml): comparisons between groups at baseline

P value (2-tailed)	n	Test statistic	Degrees of freedom
0.018*	97	8.005	2

Abbreviations: IL- 4 – Interleukin 4, pg/ml – picograms per millilitre, n – number of subjects

***** p < 0.05 compared to Week 0

3.3.3.5.3 Interleukin 10 (IL-10): comparisons between groups

Independent-samples Kruskall-Wallis test showed significant differences between groups (p = 0.021*) (see Tables 37 and 38). Plasma IL-10 levels at baseline were higher in the no acupuncture group than in the sham acupuncture group.

Table 37. IL-10 in plasma (pg/ml) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
33	23.09±5.39	31	10.91±5.17	32	20.39±9.33

Abbreviations: IL-10 – interleukin 10, pg/ml – picograms per millilitre, n – number of subjects, SE –standard error, SD – standard deviation

Table 38. IL- 10 in plasma (pg/ml): comparisons between groups at baseline

P value (2-tailed)	n	Test statistic	Degrees of freedom
0.021*	99	7.743	2

Abbreviations: IL- 10 – Interleukin 10, pg/ml – picograms per millilitre, n – number of subjects

***** p < 0.05 compared to Week 0

3.3.3.5.4 Interleukin 12(p70) (IL-12 [p70]): comparisons between groups

Independent-samples Kruskall-Wallis test showed significant differences between groups (p = 0.022*) (see Tables 39 and 40). Plasma IL-12(p70) levels at baseline were higher in the no acupuncture group than in both the real acupuncture group and the sham acupuncture group.

Table 39. IL-12(p70) in plasma (pg/ml) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
34	164.34±124.80	34	7.44±4.31	32	17.11±10.05

Abbreviations: IL-12(p70) – interleukin 12(p70), pg/ml – picograms per millilitre, n – number of subjects, SE – standard error, SD – standard deviation

Table 40. IL-12(p70) in plasma (pg/ml): comparisons between groups at baseline

P value (2-tailed)	n	Test statistic	Degrees of freedom
0.022*	104	7.652	2

Abbreviations: IL- 12(p70) – Interleukin 12(p70), pg/ml – picograms per millilitre, n – number of subjects

***** p < 0.05 compared to Week 0

3.3.3.5.5 Interferon gamma (IFNγ): comparisons between groups

There was a significant difference in plasma IFNγ levels between groups at baseline, with both the no acupuncture group and the real acupuncture higher than the sham acupuncture group (p = 0.033* and p = 0.041* respectively) (see Tables 41 and 42).

Table 41. IFNγ in plasma (pg/ml) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
35	55.77±23.43	35	11.49±2.82	34	35.46±9.14

Abbreviations: IFNγ – interferon gamma, pg/ml – picograms per millilitre, n – number of subjects, SE – standard error, SD – standard deviation

Table 42. IFNγ in plasma (pg/ml): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
-0.01 (-0.36 to 0.33)	0.935	0.32 (0.01 to 0.63)	0.041*	-0.33 (-0.64 to -0.03)	0.033*

Abbreviations: IFNγ – interferon gamma, pg/ml – picograms per millilitre, 95% CI – 95% confidence interval

3.3.3.5.6 Eotaxin: comparisons between groups

There were no significant differences in plasma eotaxin levels between groups at baseline (see Tables 43 and 44).

Table 43. Eotaxin in plasma (pg/ml) at baseline

No acupuncture		Sham acupuncture		Real acupuncture	
n	Mean (±SE)	n	Mean (±SE)	n	Mean (±SE)
35	69.54±6.46	34	65.03±9.33	34	58.57±6.75

Abbreviations: pg/ml – picograms per millilitre, n – number of subjects, SE – standard error, SD – standard deviation

Table 44. Eotaxin in plasma (pg/ml): comparisons between groups at baseline (logged data)

Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
-0.11 (-0.24 to 0.03)	0.112	-0.06 (-0.20 to 0.09)	0.435	-0.05 (-0.18 to 0.08)	0.417

Abbreviations: pg/ml – picograms per millilitre, 95% CI – 95% confidence interval

3.4 Discussion

There were no significant differences between groups at baseline (Week 0) in the demographic measures of gender, age and symptom severity, or in clinical examinations of mean inferior turbinate obstruction and peak nasal inspiratory flow. However since the gender and age distributions in the recruitment did not match the typical distributions of gender and age for allergic rhinitis sufferers in the wider Australian community, this may have produced some selection bias. Possible selection bias is discussed in Section 4.4.4 Limitations.

At baseline (Week 0) there were also no significant between-group differences in serum levels of IgE (total and allergen-specific IgE for three grass pollens and dust mite), or in plasma levels of neurotrophins and CGRP, or in saliva levels of ECP. Salivary levels of SP and VIP collected before treatment in Week 1 also showed no significant between-group differences.

There were no significant differences between groups at Week 0 in plasma eotaxin.

However, there were significant differences at baseline between groups in IL-2, IL-4, IL-10, IL-12(p70) and IFNγ. The no acupuncture group showed significantly higher levels of IL-2, IL-4, IL-10, IL-12(p70) and IFNγ than the sham acupuncture group. For IL-2 and IL-12(p70) the no acupuncture group also showed higher levels than the real acupuncture group. For IFNγ the real acupuncture group also showed higher levels than the sham group.

3.5 Conclusions

These results indicate that the no acupuncture, sham acupuncture and real acupuncture groups were well-matched at baseline in demographic and clinical characteristics. Between-group differences in cytokines at baseline is unlikely to have had any significant impact on the results of the study, as there were no significant differences in any of these cytokines between Week 0 and Week 12.

Chapter 4. A randomised sham-controlled subject-and-assessor-blinded three arm clinical trial

4.1 Abstract

Background: In a randomised sham-controlled subject-and-assessor-blinded three arm clinical trial conducted in Southport, Queensland, between October 2009 and August 2012, 151 adults with persistent allergic rhinitis were randomised into three groups – real acupuncture, sham acupuncture and no acupuncture. Each participant was involved in the trial for a period of 13 weeks, one week for screening and baseline assessment (Week 0), then 8 weeks of treatment and four weeks follow-up (Weeks 1 to 12).

Objectives: The primary outcome for the study was to measure any potential modulation of the mucosal immune response in the upper respiratory tract by acupuncture in subjects with allergic rhinitis. The secondary outcome was to measure any potential reduction in markers of mucosal inflammation (nasal airway resistance, symptoms and ECP) and improved quality of life scores following acupuncture.

Methods: Clinical examinations for each participant, namely peak nasal inspiratory flow (PNIF) and rhinoscopic examination to assess inferior turbinate obstruction, were conducted at baseline and at one-week and four-week follow-up (Weeks 0, 9 and 12). Two questionnaires were also completed at the same time-points, namely the Mini-Rhinoconjunctivitis Quality of Life Questionnaire (MiniRQLQ) and the Instantaneous Total Nasal Symptom Score (iTNSS).
Each participant also recorded daily symptoms and use of relief medications in a daily symptom and medication diary from Week 1 (first week of treatment) to Week 12.

Results: There were no significant changes in peak nasal inspiratory flow or mean inferior turbinate obstruction after acupuncture. Significant improvements in clinical symptoms and quality of life were seen after real acupuncture, and in some cases, after sham acupuncture, but no improvements were noted in the no acupuncture group.

Conclusions: Acupuncture is safe and effective in improving clinical symptoms and quality of life in adults with persistent allergic rhinitis, as well as reducing the use of relief medications. Acupuncture had no impact on objective measures of nasal patency.

4.2 Introduction

A randomised sham-controlled subject-and-assessor-blinded three arm clinical trial was conducted in Southport, Queensland, between October 2009 and August 2012. After screening, eligible subjects were randomised into three groups – real acupuncture, sham acupuncture and no acupuncture. Section 4.4 describes the flow of participants through the trial, withdrawals and exclusions, the limitations of the trial and adverse effects. Section 4.5 details the results of clinical examinations and symptom and quality of life questionnaires. Correlations between nasal patency measurements and self-reporting of sensations of nasal obstruction, as well as between symptoms and medication use are explored in Section 4.6. The remaining sections contain discussion of the significance of the results and conclusions.

4.3 Methods

Clinical examinations for each participant in the no acupuncture, sham acupuncture and real acupuncture groups were conducted at the allergy clinic at Week 0,Week 9 and Week 12 . PNIF and rhinoscopic examination to assess inferior turbinate obstruction were conducted, and subjects also filled out the MiniRQLQ and the iTNSS questionnaires.

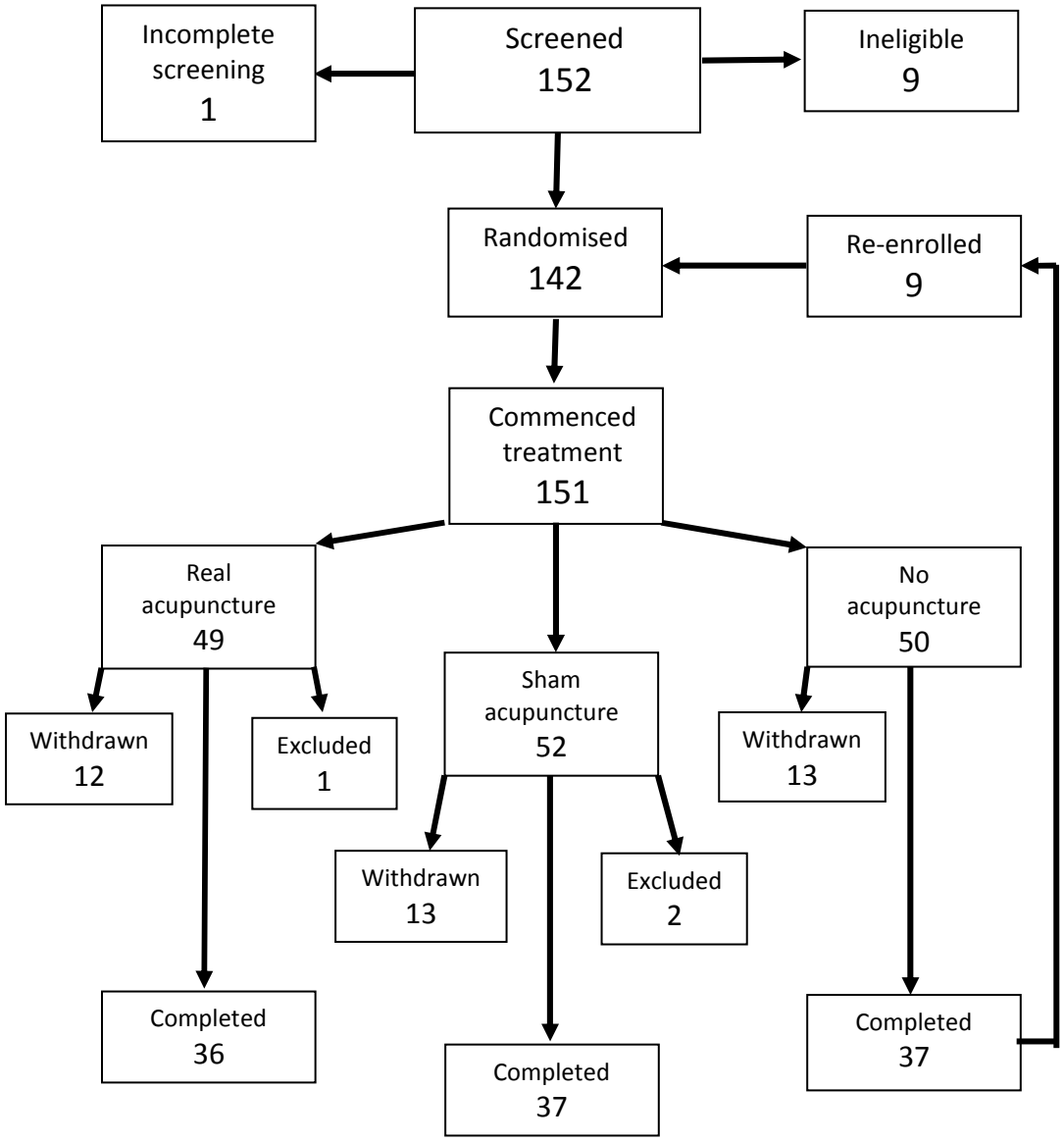
Symptoms and use of relief medications were recorded by each participant in a daily symptom and medication diary from Week 1 (first week of treatment) to Week 12. For all subjects in the real and sham acupuncture treatment groups, a third questionnaire, an exit debrief questionnaire, was also completed after the final acupuncture treatment in Week 8. For details of testing see Chapter 2.

4.4 Clinical trial

4.4.1 Participant flow

A total of 152 participants were screened however 9 were found to be ineligible and 1 did not complete the screening procedures (see Figure 1). Hence 142 participants were randomised to one of the three study groups - real acupuncture, sham acupuncture or no acupuncture. Upon completion of the study in the no acupuncture group, participants were invited to re-enrol in the study and be randomised to either the real or sham acupuncture group. A total of 9 participants who completed the study in no acupuncture group opted to re-enrol (see Figure 1). The study was completed by 110 participants, while 38 withdrew and 3 were excluded (see Figure 1). The numbers of participants who completed the study in each group met the required sample size of 36 per group. However the number of withdrawals exceeded 20% (38/148 = 25.6%).

Figure 1. Participant flow chart



4.4.2 Withdrawals and exclusions

There were a total of 38 withdrawals from the study (see Tables 1 and 2), and 3 exclusions due to protocol violations. Of the 38 withdrawals, 16 participants withdrew prior to their first attendance at the acupuncture clinic (see Table 2).

Table 1. Withdrawals from the study

Acupuncture group	Week	Reason given
No	Wk 0	None
No	Wk 0	Time constraints
No	Wk 0	Work hours prevent participation
No	Wk 0	No show – not responding to phone calls
No	Wk 0	None
No	Wk 0	Extremely busy; only interested if in treatment group
No	Wk 0	None
No	Wk 0	No show – not responding to phone calls
No	Wk 3	Unforeseen personal problems
No	Wk 3	"Going away for a few months"
No	Wk 6	Have to go overseas for a few weeks
No	Wk 9	Very busy at work
No	Wk 9	Did not attend Wk 9 Follow-up
Sham	Wk 0	None
Sham	Wk 0	Unforeseen family health and medical issues
Sham	Wk 0	Unable to keep appointments due to work
Sham	Wk0	Change of circumstances
Sham	Wk 0	Travel
Sham	Wk 1	New job requiring travel
Sham	Wk 1	Sensitive to moxa smoke smell in clinic
Sham	Wk 4	None
Sham	Wk 4	Interstate job
Sham	Wk 6	Unforeseen circumstances
Sham	Wk 6	Unable to keep appointments
Sham	Wk 7	No show – not responding to phone calls
Sham	Wk 7	Unable to keep appointments due to work
Real	Wk 0	None
Real	Wk 0	Travel
Real	Wk 0	Schedule too full to attend morning appts
Real	Wk 0	Unable to arrange transport
Real	Wk 1	Needle phobia
Real	Wk 1	Changed work hours and unable to keep appointments
Real	Wk 2	Personal reasons
Real	Wk 3	Unable to keep appointments
Real	Wk 3	No show – not responding to phone calls
Real	Wk 4	Moved away from Gold Coast due to family issues
Real	Wk 7	No show – not responding to phone calls
Real	Wk 9	Did not attend Wk 9 Follow-up

Table 2. Withdrawals by group and by week

	Real acupuncture	Sham acupuncture	No acupuncture	n
Wk 0	3	5	8	**16***
Wk 1	2	2		**4**
Wk 2	1			**1**
Wk 3	3		2	**5**
Wk 4	1	2		**3**
Wk 6		2	1	**3**
Wk 7	1	2		**3**
Wk 9	1		2	**3**
	12	**13**	**13**	**38**

*Not seen at acupuncture clinic

There were three exclusions from the study due to protocol violations. One subject was commenced on medications for asthma and depression, a second subject was commenced on medications for asthma, and a third subject was commenced on anti-depressants. These medications constitute exclusion criteria for the study.

4.4.3 Adverse events

There were no serious adverse events reported or observed during the study. There were 40 minor adverse events during the study, mainly slight bruising (27), and acute transitory pain on needle insertion (8) (see Table 3). As approximately 1,300 acupuncture treatments were administered during the study, this represents a minor adverse events rate of 3%.

Table 3. Adverse events

Slight bruising	27
Acute transitory pain on insertion	7
Acute transitory pain and pins and needles sensation on insertion	1
Soreness	2
Soreness and itching	1
Swellling	1
Tingling	1
Total minor adverse events	**40**

4.4.4 Limitations

4.4.4.1 Gender bias

There was a strongly female gender bias in the numbers of subjects screened (111/152 or 73.0% female) and the number of subjects who completed the trial (81/110 or 73.6% female). As the sample for this study was a “convenience sample” - consisting of those who responded to the staff/student emails sent out at Griffith University, newspaper and

television coverage of the study and other advertising – this gender bias simply reflects the population who volunteered for the study. Although no formal statistics are available, anecdotal evidence from Australian acupuncturists suggests that the population of acupuncture patients in Australia is generally around 70% female. So it is possible that this gender bias in our sample represents a greater acceptance of acupuncture treatment by women. This does not reflect the gender balance of adults with allergic rhinitis in the Australian community. The 2011-2012 Australian Bureau of Statistics Australian Health Survey estimated the ratio of females to males with allergic rhinitis in Australia (all age groups included) was 1,918,600:1,783,200 (51.8% female).

4.4.4.2 Age bias

Since only adults between 18 and 45 years were selected for the study, the results cannot be generalised to the Australian community at large. As the number of Australians with allergic rhinitis in the age brackets from 15 to 44 years is only 63.2% of the total population of allergic rhinitis sufferers, the study sample recruited did not accurately reflect all age groups (see shaded areas of Table 4) (ABS, 2013).

Table 4. Australian Health Survey: First Results, 2011–12 — Australia

	Age group (years) (%)							
	0–14	15–24	25–34	35–44	45–54	55–64	65–74	75 years and over
Hayfever and allergic rhinitis	9.5%	18.8%	21.9%	22.5%	18.9%	14.1%	14.3%	12.1%

4.4.4.3 Randomisation bias

A total of 9 subjects who completed the trial in the no acupuncture group were re-enrolled and randomised to either the real or sham acupuncture groups (but not to the no acupuncture group for a second time). Of these 9 re-enrolled subjects, 7 completed the second course of treatment in the study (6.4% of completed subjects). Due to the small number of subjects involved, this randomisation bias would have been unlikely to have a large impact on final results.

4.4.4.4 Disease bias

Since asthma sufferers who also had allergic rhinitis were excluded from the study, this would produce a disease bias in the sample. The clinical connections between allergic

rhinitis and asthma have been highlighted by the ARIA (Allergic Rhinitis and its Impact on Asthma) group (Bousquet et al., 2008; Braunstahl, 2009; Togias, 2003). It has been reported that 40% of the allergic rhinitis patients have lower airways involvement, while 80-95% of allergic asthma patients have concomitant rhinitis (Braunstahl, 2009). Allergic rhinitis has also been identified as a risk factor in the development of adult-onset asthma, increasing risk by 3-5 times (Guerra, 2002).

4.4.4.5 Sample size

Although the sample size which was calculated in the power calculation for the study was achieved (110 with 108 required), the drop-out rate exceeded 20%, namely 25.6%. Since the power calculation was based on what sample size would be needed to detect improvement in clinical symptoms (which was achieved), it is possible that the study was under-powered to detect potential changes in biomarkers such as neurotrophins and cytokines. The sample size was adequate, however, to show significant differences in immunoglobulins and some neuropeptides. For details of the power calculation see section 2.3.5.

4.5 Results

Outcome measures for the study included clinical examinations, self-reporting tools and laboratory biomarkers (measured in blood serum, blood plasma and saliva). The results from clinical assessments and self-reporting tools are covered in this chapter, while the results from laboratory tests are covered in Chapter 5. Methods are detailed in Chapter 2.

4.5.1 Clinical examinations

Objective measurement of nasal patency was undertaken using peak nasal inspiratory flow and rhinoscopic examination of nasal obstruction at the level of the inferior turbinates. PNIF and mean inferior turbinate obstruction were measured at the allergy clinic during each visit in Week 0, 9 and 12.

4.5.1.1 Peak nasal inspiratory flow

4.5.1.1.1 Time-point comparisons within groups

PNIF scores were compared between baseline (Week 0), one-week follow-up (Week 9) and four-week follow-up (Week 12) for each group. The data was normally distributed on both Kolgomorov-Smirnov and Shapiro-Wilk tests for normality.

Paired T tests were used to compare time-points within each group. There were no significant differences in any group between Week 0 and Week 9 or between Week 0 and Week 12, except for a significant difference between Week 0 and Week 12 in the no acupuncture group (p = 0.016*) (see Tables 5 and 6). This change was not related to the effects of acupuncture treatment, as this group received no acupuncture treatment.

Table 5. Peak nasal inspiratory flow (litres/minute): group means with standard errors

	No acupuncture (n = 23)	Sham acupuncture (n = 26)	Real acupuncture (n =22)
Week	Mean (±SE)	Mean (±SE)	Mean (±SE)
0	70.2±5.2	67.9±6.0	84.3±7.0
9	77.8±6.7	70.8±4.2	77.6±7.3
12	90.7±7.1*	72.5±4.7	88.2±6.8

Abbreviations: n – number of subjects, SE – standard error, SD – standard deviation

* p < 0.05 compared to Week 0, ** p<0.001 compared to Week 0

Table 6. Peak nasal inspiratory flow (litres/minute): time-point differences within groups

	Week 0 vs Week 9			Week 0 vs Week 12		
Group	n	Difference (95% CI)	P value	n	Difference (95% CI)	P value
Real acupuncture	30	-0.0 (-9.9 to 9.9)	0.997	26	-5.6 (-15.6 to 4.4)	0.261
Sham acupuncture	31	-5.8 (-19.0 to 7.3)	0.374	30	-4.0 (-17.0 to 9.0)	0.535
No acupuncture	29	-0.2 (-12.7 to 12.3)	0.682	26	-17.3 (-31.1 to -3.5)	0.016*

Abbreviations: n – number of subjects, 95% CI – 95% confidence interval

4.5.1.1.2 Time-point differences between groups

Independent samples T tests were used to compare groups at each time-point. There were no significant differences between groups (see Table 7).

Table 7. Peak nasal inspiratory flow (litres/minute): time-point differences between groups

	Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Week	Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0	-2.0 (-17.8 to 13.8)	0.801	3.6 (-12.1 to 19.3)	0.651	-5.6 (-22.4 to 11.2)	0.551
9	2.2 (-13.8 to 18.2)	0.786	1.4 (-13.6 to 16.5)	0.849	0.7 (-14.5 to 15.9)	0.923
12	-5.9 (-23.9 to 12.0)	0.510	8.0 (-8.2 to 24.3)	0.327	-14.0 (-31.1 to 3.1)	0.107

Abbreviations: 95% CI – 95% confidence interval

4.5.1.2 Mean inferior turbinate obstruction

4.5.1.2.1 Time-point comparisons within groups

Mean (between left and right) inferior turbinate obstruction scores (expressed as a percentage of the obstruction of the airways at the level of the inferior turbinates) were measured by rhinoscopic examination by the allergologist at baseline (Week 0), one-week follow-up (Week 9) and four-week follow-up (Week 12) for each group. Paired T tests were used to compare time-points for each group (as the data was normally distributed on both Kolgomorov-Smirnov and Shapiro-Wilk tests for normality). There were no significant differences in any group between time-points (see Tables 8 and 9).

Table 8. Mean inferior turbinate obstruction (%): group means with standard errors

	No acupuncture (n = 32)	Sham acupuncture (n = 34)	Real acupuncture (n = 35)
Week	Mean (±SE)	Mean (±SE)	Mean (±SE)
0	69±3.3	66±3.0	67±2.7
9	71±3.0	63±3.2	64±3.1
12	64±2.6	66±3.2	62±3.3

Table 9. Mean inferior turbinate obstruction (%): time-point differences within groups

Group	Week 0 vs Week 9			Week 0 vs Week 12		
	n	Difference (95% CI)	P value	n	Difference (95% CI)	P value
Real acupuncture	35	3.2 (-3.2 to 9.7)	0.319	36	4.9 (-0.75 to 10.5)	0.087
Sham acupuncture	35	3.9 (-3.2 to 11.0)	0.270	36	0.9 (-6.8 to 8.6)	0.812
No acupuncture	34	-1.7 (-10.0 to 6.6)	0.682	33	4.1 (-4.5 to 12.7)	0.340

Abbreviations: n – number of subjects, 95% CI – 95% confidence interval

4.5.1.2.2 Time-point differences between groups

Independent samples T tests were used to compare groups at each time-point. The only significant between-group difference was between the sham acupuncture and no acupuncture groups at Week 9 ($p = 0.019^*$) (see Table 10).

Table 10. Mean inferior turbinate obstruction (%): time-point differences between groups

Week	Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
	Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0	1.7 (-6.1 to 9.6)	0.661	-2.4 (-10.4 to 5.5)	0.544	4.2 (-4.3 to 12.6)	0.331
9	7.9 (-0.6 to 16.3)	0.067	-2.6 (-11.8 to 6.5)	0.565	10.5 (1.8 to 19.2)	0.019*
12	2.4 (-5.7 to 10.6)	0.557	2.6 (-6.2 to 11.4)	0.552	-0.2 (-8.2 to 7.8)	0.958

Abbreviations: 95% CI – 95% confidence interval

* $p < 0.05$ compared to Week 0

4.5.2 Questionnaires and daily diaries

Instantaneous total nasal symptom scores (iTNSS) and MiniRQLQ were administered at the allergy clinic during each visit in Week 0, 9 and 12. The daily symptom and medication dairy was recorded daily by each participant.

4.5.2.1 Instantaneous total nasal symptom scores (iTNSS)

4.5.2.1.1 Time-point comparisons within groups

Total iTNSS scores were compared between baseline (Week 0), one-week follow-up (Week 9) and four-week follow-up (Week 12) for each group. Paired T tests were used to compare time-points for each group (as the data was normally distributed on both Kolgomorov-Smirnov and Shapiro-Wilk tests for normality). In the real acupuncture group there was a significant difference between Week 0 and Week 9 ($p = 0.010^*$), and between Week 0 and Week 12 ($p = 0.003^*$) (see Tables 11 and 12).

Table 11. Instantaneous total nasal symptom scores (iTNSS): group means with standard errors

	No acupuncture (n = 37)	Sham acupuncture (n = 37)	Real acupuncture (n = 36)
Week	Mean (±SE)	Mean (±SE)	Mean (±SE)
0	4.8±0.5	5.0±0.4	5.4±0.5
9	4.3±0.3	4.2±0.5	3.9±0.5*
12	4.3±0.4	4.3±0.5	3.6±0.5*

Abbreviations: n – number of subjects, SE – standard error, SD – standard deviation

* $p < 0.05$ compared to Week 0

Table 12. Instantaneous total nasal symptom scores (iTNSS): time-point differences within groups

Group	Week 0 vs Week 9			Week 0 vs Week 12		
	n	Difference (95% CI)	P value	n	Difference (95% CI)	P value
Real acupuncture	36	1.5 (0.4 to 2.7)	0.010*	36	1.9 (0.7 to 3.1)	0.003*
Sham acupuncture	37	0.8 (-0.3 to 2.0)	0.147	37	0.7 (-0.5 to 1.9)	0.257
No acupuncture	37	0.5 (-0.4 to 1.4)	0.259	37	0.5 (-0.4 to 1.5)	0.244

Abbreviations: n – number of subjects, 95% CI – 95% confidence interval

4.5.2.1 Time-point differences between groups

Independent samples T tests were used to compare groups at each time-point. There were no significant differences between groups (see Table 13).

Table 13. Instantaneous total nasal symptom scores (iTNSS): time-point differences between groups

	Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Week	Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0	-0.6 (-2.0 to 0.7)	0.373	-0.4 (-1.6 to 0.8)	0.531	-0.2 (-1.4 to 0.96)	0.715
9	0.4 (-0.7 to 1.6)	0.478	0.3 (-1.1 to 1.7)	0.660	0.1 (-1.1 to 1.3)	0.859
12	0.7 (-0.5 to 1.9)	0.251	0.8 (-0.6 to 2.1)	0.260	-0.1 (-1.3 to 1.2)	0.932

Abbreviations: 95% CI – 95% confidence interval

4.5.2.2 Mini Rhinoconjunctivitis Quality of Life Questionnaire (MiniRQLQ)

4.5.2.2.1 Mini Rhinoconjunctivitis Quality of Life Questionnaire (MiniRQLQ) – Total scores

4.5.2.2.1.1 Time-point comparisons within groups

Total MiniRQLQ scores were compared between baseline (Week 0), one-week follow-up (Week 9) and four-week follow-up (Week 12) for each group. Paired T tests were used to compare time-points for each group (as the data was normally distributed on both Kolgomorov-Smirnov and Shapiro-Wilk tests for normality). There were significant differences between Week 0 and Week 9 in the sham acupuncture group (p = 0.001**) and the real acupuncture group (p = 0.001**) (see Table 15). There were also significant differences between Week 0 and Week 12 in the sham acupuncture group (p = 0.007*) and the real acupuncture group (p = 0.001**) (see Table 15).

Table 14. MiniRQLQ total scores: group means with standard errors

	No acupuncture (n = 37)	Sham acupuncture (n = 37)	Real acupuncture (n = 36)
Week	Mean (±SE)	Mean (±SE)	Mean (±SE)
0	44±2.6	47±2.1	44±2.7
9	44±2.0	35±3.0**	34±3.1**
12	41±2.7	39±3.2*	31±2.9**

Abbreviations: n – number of subjects, SE – standard error, SD – standard deviation

* p < 0.05 compared to Week 0, ** p<0.001 compared to Week 0

Table 15. MiniRQLQ total scores: time-point differences within groups

Group	Week 0 vs Week 9			Week 0 vs Week 12		
	n	Difference (95% CI)	P value	n	Difference (95% CI)	P value
Real acupuncture	36	10.3 (4.7 to 15.9)	0.001**	36	13.6 (7.7 to 19.5)	0.001**
Sham acupuncture	37	12.4 (6.6 to 18.1)	0.001**	37	8.2 (2.4 to 13.9)	0.007*
No acupuncture	37	-0.8 (-6.3 to 4.7)	0.766	37	2.8 (-4.2 to 9.9)	0.419

Abbreviations: MiniRQLQ – Mini Rhinoconjunctivitis Quality of Life Questionnaire, n – number of subjects, 95% CI – 95% confidence interval

* $p < 0.05$ compared to Week 0, ** $p<0.001$ compared to Week 0

4.5.2.2.1.2 Time-point differences between groups

Independent samples T tests were used to compare groups at each time-point. There were significant differences between the real acupuncture group and the no acupuncture group at Week 9 ($p = 0.006$*) and Week 12 ($p = 0.014$*), and between the sham acupuncture group and the no acupuncture group at Week 9 ($p = 0.007$*) (see Table 16).

Table 16. MiniRQLQ total scores: time-point differences between groups

Week	Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
	Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0	-0.7 (-8.2 to 6.8)	0.850	2.6 (-4.3 to 9.4)	0.458	-3.3 (-9.9 to 3.4)	0.330
9	10.4 (3.1 to 17.7)	0.006*	0.5 (-8.1 to 9.1)	0.916	9.9 (2.8 to 17.1)	0.007*
12	10.1 (2.1 to 18.0)	0.014*	8.0 (-0.6 to 16.6)	0.068	2.1 (-6.3 to 10.4)	0.624

Abbreviations: 95% CI – 95% confidence interval

* $p < 0.05$ compared to Week 0

4.5.2.2.2 MiniRQLQ - Domain scores

4.5.2.2.2.1 Time-point comparisons within groups

MiniRQLQ scores were divided into 5 domains: activities, practical problems, nose symptoms, eye symptoms and other symptoms. Each of the domain scores were compared between baseline (Week 0), one week follow-up (Week 9) and four-week follow-up (Week 12) for each group. Paired T tests were used to compare time-points for each group (as the data was normally distributed on both Kolgomorov-Smirnov and Shapiro-Wilk tests for normality). There were significant differences between Week 0 and Week 9 in the real acupuncture group for all five domains except "other symptoms", and in the sham acupuncture group, for all domains (see Table 18). There were significant differences between Week 0 and Week 12 in the real acupuncture

group for all five domains and in the sham acupuncture group for all domains except "other symptoms" (see Table 18).

Table 17. MiniRQLQ domain scores: group means with standard errors

		No acupuncture (n = 37)	Sham acupuncture (n = 37)	Real acupuncture (n = 36)
MiniRQLQ domain score	Week	Mean (±SE)	Mean (±SE)	Mean (±SE)
Activities	0	9±0.6	10±0.5	9±0.6
	9	9±0.6	7±0.6**	7±0.6*
	12	9±0.6	8±0.7*	6±0.6**
Practical problems	0	7±0.5	8±0.4	8±0.5
	9	7±0.4	6±0.6**	6±0.5**
	12	6±0.5	7±0.6*	5±0.5**
Nasal symptoms	0	10±0.7	11±0.6	11±0.7
	9	10±0.7	9±0.8**	8±0.7**
	12	9±0.7	10±0.8*	8±0.7**
Eye symptoms	0	9±0.8	10±0.7	9±0.7
	9	9±0.7	6±0.8**	6±0.8**
	12	8±0.7	7±0.8*	6±0.8*
Other symptoms	0	8±0.6	8±0.7	8±0.7
	9	9±0.6	7±0.8*	7±0.8
	12	9±0.7	8±0.8	6±0.7*

Abbreviations: n – number of subjects, SE – standard error, SD – standard deviation

* $p < 0.05$ compared to Week 0, ** $p<0.001$ compared to Week 0

Table 18. MiniRQLQ domain scores: time-point differences within groups

Group	Domain	Week 0 vs Week 9		Week 0 vs Week 12	
		Difference (95% CI)	P value	Difference (95% CI)	P value
Real acupuncture (n = 36)	Activities	1.9 (0.8 to 3.1)	0.002*	2.6 (1.3 to 3.8)	0.001**
	Practical problems	2.1 (1.0 to 3.1)	0.001**	2.7 (1.5 to 3.9)	0.001**
	Nasal symptoms	2.8 (1.2 to 4.4)	0.001**	3.6 (1.9 to 5.4)	0.001**
	Eye symptoms	2.5 (1.0 to 4.0)	0.001**	2.7 (1.1 to 4.3)	0.002*
	Other symptoms	1.0 (-0.2 to 2.2)	0.112	2.0 (0.7 to 3.2)	0.003*
Sham acupuncture (n = 37)	Activities	2.3 (1.1 to 3.5)	0.001**	1.8 (0.4 to 3.2)	0.014*
	Practical problems	2.0 (0.9 to 3.0)	0.001**	1.2 (0.2 to 2.2)	0.016*
	Nasal symptoms	2.9 (1.6 to 4.2)	0.001**	1.8 (0.4 to 3.2)	0.011*
	Eye symptoms	3.7 (2.1 to 5.3)	0.001**	2.6 (0.8 to 4.4)	0.006*
	Other symptoms	1.4 (0.0 to 2.9)	0.048*	0.7 (-0.8 to 2.2)	0.362
No acupuncture (n = 37)	Activities	-0.8 (-2.0 to 0.5)	0.226	0.0 (-1.5 to 1.5)	1.000
	Practical problems	0.2 (-0.9 to 1.3)	0.765	0.9 (-0.4 to 2.2)	0.166
	Nasal symptoms	0.1 (-1.4 to 1.6)	0.887	0.7 (-1.0 to 2.4)	0.421
	Eye symptoms	0.6 (-1.0 to 2.2)	0.444	1.4 (-0.4 to 3.2)	0.126
	Other symptoms	- 0.9 (-2.2 to 0.4)	0.156	-0.1 (-1.9 to 1.6)	0.875

Abbreviations: MiniRQLQ – Mini Rhinoconjunctivitis Quality of Life Questionnaire, n – number of subjects, 95% CI – 95% confidence interval

* $p < 0.05$ compared to Week 0, ** $p<0.001$ compared to Week 0

4.5.2.2.2.2 Time-point differences between groups

Independent samples T tests were used to compare groups at each time-point. There were significant differences between the real acupuncture group and the no acupuncture group in Week 9 for activities total score (p = 0.001**), eye symptoms total score (p = 0.022*) and other symptoms total score (p = 0.014*), and in Week 12 for activities total score (p = 0.003*) and other symptoms total score (p = 0.007*) (see Table 19). There were significant differences between the sham acupuncture and the no acupuncture

group at Week 9 for activities total score (p = 0.005*), eye symptoms total score (p = 0.008 *) and other symptoms total score (p = 0.018*) (see Table 19).

Table 19. MiniRQLQ domain scores: time-point differences between groups

	Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
	Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
Week 0						
Activities	0.1 (-1.6 to 1.7)	0.939	0.9 (-0.7 to 2.4)	0.268	-0.8 (-2.3 to 0.7)	0.284
Practical problems	-0.6 (-2.0 to 0.8)	0.391	0.0 (-1.3 to 1.3)	0.996	-0.6 (-1.9 to 0.7)	0.349
Nasal symptoms	-1.3 (-3.3 to 0.8)	0.231	0.2 (-1.6 to 2.0)	0.821	-1.5 (-3.3 to 0.4)	0.117
Eye symptoms	0.5 (-1.6 to 2.7)	0.608	0.9 (-1.0 to 2.9)	0.343	-0.4 (-2.4 to 1.7)	0.716
Other symptoms	0.5 (-1.3 to 2.4)	0.553	0.5 (-1.3 to 2.4)	0.565	0.0 (-1.9 to 1.9)	1.000
Week 9						
Activities	-2.8 (-4.3 to -1.2)	0.001**	-0.5 (-2.2 to 1.3)	0.576	2.3 (0.7 to 3.8)	0.005*
Practical problems	-1.3 (-2.6 to 0.0)	0.053	-0.1 (-1.6 to 1.4)	0.910	1.2 (-0.2 to 2.6)	0.093
Nasal symptoms	-1.4 (-3.3 to 0.5)	0.137	-0.0 (-2.2 to 2.1)	0.909	1.4 (-0.6 to 3.4)	0.179
Eye symptoms	-2.5 (-4.5 to -0.4)	0.022*	0.3 (-1.9 to 2.5)	0.806	2.7 (0.7 to 4.7)	0.008*
Other symptoms	-2.5 (-4.4 to -0.5)	0.014*	-0.1 (-2.3 to 2.1)	0.920	2.4 (0.4 to 4.3)	0.018*
Week 12						
Activities	-2.6 (-4.3 to-0.9)	0.003*	-1.6 (-3.4 to 0.1)	0.066	1.0 (-0.8 to 2.7)	0.277
Practical problems	-1.2 (-2.6 to 0.1)	0.077	-1.5 (-3.0 to 0.1)	0.060	-0.3 (-1.8 to 1.2)	0.721
Nasal symptoms	-1.7 (-3.7 to 0.3)	0.092	-2.0 (-4.2 to 0.1)	0.066	-0.3 (-2.4 to 1.8)	0.779
Eye symptoms	-1.9 (-3.9 to 0.2)	0.077	-1.0 (-3.3 to 1.2)	0.362	0.8 (-1.4 to 3.0)	0.449
Other symptoms	-2.7 (-4.6 to -0.7)	0.007*	-1.8 (-4.0 to 0.3)	0.091	0.8 (-1.3 to 2.9)	0.447

Abbreviations: 95% CI – 95% confidence interval

* p < 0.05 compared to Week 0, ** p<0.001 compared to Week 0

4.5.2.3 Daily symptom and medication diaries

Daily symptom and medication diaries were recorded by participants starting at Week 1 and finishing at Week 12. Participants were asked to record the presence of each of seven symptoms: nasal itch, eye itch, sneezing, runny nose, post-nasal drip, unrefreshed sleep and sinus pain. This yielded weekly total symptom scores ranging from 0 to 49. All medications taken during the trial were also recorded daily. Of the 110 participants who completed the study, 88 returned their daily symptom and medication diaries.

4.5.2.3.1 Daily symptom diary weekly total scores

4.5.2.3.1.1 Time-point comparisons within groups

Paired T tests were used to compare time-points for each group (as the data was normally distributed using the Kolgomorov-Smirnov test for normality). In the real acupuncture group there were significant differences between total weekly symptom scores for Week 1 and Week 3 ($p = 0.023^*$) and significant differences in total weekly symptoms scores persisted every week until Week 12 (see Table 21). There were no significant differences between Week 1 and any other week in the other groups (see Table 21).

Table 20. Daily symptom diary mean weekly total scores: group means with standard errors

	No acupuncture (n = 27)	Sham acupuncture (n = 31)	Real acupuncture (n = 30)
Week	Mean (±SE)	Mean (±SE)	Mean (±SE)
1	19±2.4	19±1.9	23±2.7
2	19±2.7	20±1.9	22±2.9
3	19±2.7	19±1.9	20±3.0
4	19±2.6	17±2.2	19±3.1
5	20±2.8	16±2.3	18±3.2
6	19±2.6	15±2.2	18±3.1
7	19±2.6	17±2.2	19±3.1
8	17±2.7	15±1.8	18±3.1
9	17±2.8	15±2.0	19±3.2
10	18±2.6	15±2.0	19±3.2
11	19±2.9	15±2.2	18±3.2
12	19±3.1	15±2.1	18±3.4

Abbreviations: n – number of subjects, SE – standard error, N/A – not applicable

Table 21. Daily symptom diary mean weekly total scores: time-point differences within groups

Week	No acupuncture (n = 27)		Sham acupuncture (n = 31)		Real acupuncture (n = 30)	
	Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
1 vs 2	0.2 (-3.2 to 3.6)	0.911	-0.5 (-4.1 to 3.1)	0.787	1.5 (-0.4 to 3.5)	0.123
1 vs 3	0.6 (-2.8 to 3.9)	0.738	0.2 (-4.1 to 4.6)	0.916	2.9 (0.4 to 5.4)	0.023*
1 vs 4	0.3 (-3.7 to 4.3)	0.865	2.4 (-1.5 to 6.4)	0.220	4.3 (1.3 to 7.3)	0.007*
1 vs 5	-0.5 (-5.6 to 4.5)	0.835	2.8 (-2.0 to 7.6)	0.239	5.1 (2.3 to 7.9)	0.001**
1 vs 6	0.5 (-3.8 to 4.8)	0.807	4.4 (-0.0 to 8.9)	0.052	5.6 (2.7 to 8.5)	0.001**
1 vs 7	0.1 (-4.7 to 4.8)	0.975	2.0 (-2.5 to 6.5)	0.375	4.6 (1.7 to 7.5)	0.003*
1 vs 8	1.7 (-2.4 to 5.9)	0.401	3.6 (-0.5 to 7.7)	0.080	5.2 (2.2 to 8.2)	0.001**
1 vs 9	1.7 (-2.6 to 6.1)	0.422	3.7 (-0.5 to 7.9)	0.081	4.6 (1.2 to 8.0)	0.009*
1 vs 10	0.8 (-3.7 to 5.4)	0.717	3.6 (-0.4 to 7.6)	0.079	4.8 (1.2 to 8.4)	0.011*
1 vs 11	0.1 (-4.4 to 4.5)	0.973	3.6 (-1.3 to 8.5)	0.144	5.2 (1.8 to 8.5)	0.004*
1 vs 12	0.2 (-4.2 to 4.6)	0.918	3.7 (2.3 to 8.4)	0.114	5.7 (2.1 to 9.2)	0.003*

Abbreviations: n – number of subjects, 95% CI- 95% confidence interval

* $p < 0.05$ compared to Week 0, ** $p<0.001$ compared to Week 0

4.5.2.3.1.2 Time-point differences between groups

Independent samples T tests were used to compare groups at each time-point. There were no significant differences between groups at any time-point (see Table 22).

Table 22. Daily symptom diary mean weekly total scores: time-point differences between groups

Week	Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
	Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
1	4.1 (-3.2 to 11.3)	0.264	4.2 (-2.3 to 10.8)	0.201	-0.2 (-6.2 to 5.9)	0.960
2	2.7 (-5.3 to 10.7)	0.496	2.2 (-4.6 to 9.1)	0.520	0.5 (-6.0 to 7.0)	0.874
3	1.7 (-6.5 to 9.9)	0.678	1.5 (-5.5 to 8.5)	0.665	0.2 (-6.4 to 6.7)	0.957
4	0.1 (-8.1 to 8.4)	0.978	2.4 (-5.3 to 10.0)	0.541	-2.2 (-9.0 to 4.6)	0.512
5	-1.6 (-10.2 to 7.0)	0.715	1.9 (-5.9 to 9.7)	0.627	-3.5 (-10.6 to 3.6)	0.332
6	-1.0 (-9.3 to 7.3)	0.804	3.0 (-4.6 to 10.7)	0.433	-4.1 (-10.9 to 2.8)	0.242
7	-0.4 (-8.7 to 7.9)	0.921	1.7 (-5.9 to 9.2)	0.660	-2.1 (-8.8 to 4.6)	0.538
8	0.6 (-7.7 to 9.0)	0.882	2.6 (-4.5 to 9.8)	0.460	-2.0 (-8.3 to 4.2)	0.519
9	1.2 (-7.4 to 9.8)	0.783	3.3 (-4.2 to 10.9)	0.383	-2.1 (-8.9 to 4.6)	0.532
10	0.1 (-8.3 to 8.6)	0.976	3.0 (-4.5 to 10.6)	0.422	-2.9 (-9.5 to 3.7)	0.378
11	-1.0 (-9.8 to 7.8)	0.818	2.7 (-5.1 to 10.5)	0.495	-3.7 (-10.9 to 3.5)	0.308
12	-1.4 (-10.5 to 7.8)	0.767	2.3 (-5.6 to 10.2)	0.560	-3.7 (-10.9 to 3.6)	0.315

Abbreviations: 95% CI – 95% confidence interval

4.5.2.3.2 Daily medication diary weekly total scores

4.5.2.3.2.1 Time-point comparison within groups

As the data was not normally distributed on Kolmogorov-Smirnov and Shapiro-Wilk tests for normality, even after log transformation, Friedman's tests were used to compare medication weekly scores between weeks. The only significant difference was between Week 1 and Week 12 in the real acupuncture group (p = 0.034*) (See Table 24).

Table 23. Daily medication diary mean weekly total scores: group means with standard errors

	No acupuncture (n = 27)	Sham acupuncture (n =31)	Real acupuncture (n = 30)
Week	Mean (±SE)	Mean (±SE)	Mean (±SE)
1	3.6±0.7	1.8±0.5	2.4±0.6
2	3.6±0.6	2.4±0.5	2.5±0.6
3	3.8±0.6	2.3±0.5	2.3±0.6
4	3.6±0.6	2.1±0.5	2.4±0.6
5	3.4±0.7	2.1±0.5	2.6±0.6
6	3.6±0.6	2.2±0.5	2.3±0.6
7	3.9±0.6	2.1±0.5	2.4±0.6
8	3.9±0.7	1.8±0.5	2.3±0.6
9	4.0±0.7	2.1±0.5	2.5±0.6
10	3.7±0.7	2.1±0.5	2.7±0.6
11	3.9±0.7	1.9±0.5	2.6±0.6
12	4.1±0.6	2.2±0.5	1.9±0.6*

Abbreviations – n – number of subjects, SE – standard error

* p < 0.05 compared to Week 1

Table 24. Daily medication diary mean weekly total scores: time-point differences within groups

Week	No acupuncture (n = 27)		Sham acupuncture (n = 31)		Real acupuncture (n = 30)	
	P value	Test statistic	P value	Test statistic	P value	Test statistic
1 vs 2	1.000	0.000	0.132	2.273	1.000	0.000
1 vs 3	0.414	0.667	0.480	0.500	0.102	2.667
1 vs 4	1.000	0.000	0.564	0.333	0.739	0.111
1 vs 5	0.180	1.800	0.782	0.077	0.705	0.143
1 vs 6	1.000	0.000	1.000	0.000	0.414	0.667
1 vs 7	1.000	0.000	1.000	0.000	0.414	0.667
1 vs 8	0.414	0.667	0.763	0.091	0.739	0.111
1 vs 9	1.000	0.000	0.782	0.077	0.705	0.143
1 vs 10	0.414	0.667	0.564	0.333	0.763	0.091
1 vs 11	0.257	1.286	1.000	0.000	0.739	0.111
1 vs 12	0.705	0.143	0.593	0.286	0.034*	4.500

Abbreviations: n – number of subjects

* p < 0.05 compared to Week 1

4.5.2.3.2.2 Time-point differences between groups

Between-group comparisons for each week were performed with the Independent-Samples Kruskal-Wallis test. In Week 12 medication use was lower in the real acupuncture group than in the no acupuncture group, but not significantly different from the sham acupuncture group (p = 0.033*) (see Table 25).

Table 25. Daily medication diary mean weekly total scores: time-point differences between groups

Week	P value (2-tailed)	n	Test statistic	Degrees of freedom
1	0.151	88	3.781	2
2	0.338	88	2.168	2
3	0.111	88	4.400	2
4	0.155	88	3.733	2
5	0.458	88	1.563	2
6	0.160	88	3.659	2
7	0.066	88	5.440	2
8	0.121	88	4.216	2
9	0.097	88	4.668	2
10	0.161	88	3.649	2
11	0.118	88	4.270	2
12	0.033*	88	6.807	2

Abbreviations: n – number of subjects

4.5.3 Exit debrief questionnaire

The results from the exit debrief questionnaire indicate that subject-blinding was maintained successfully until the completion of the acupuncture treatments (see Table 26). This suggests that any effects from acupuncture treatment which might be due to positive expectation and belief (placebo response) would most likely have been uniformly distributed between the sham and real acupuncture groups.

Table 26. Exit debrief questionnaire: results for real and sham acupuncture groups

	n	Thought real group	Thought sham group	Undecided
Real acupuncture	37^	17 (45.9%)	18 (48.6%)	2 (5.4%)
Sham acupuncture	35	17 (48.6%)	16 (45.7%)	2 (5.7%)

^NB: an error has occurred in the labelling of one of the envelopes as there were only 36 participants who completed the real acupuncture group and 37 who completed the sham acupuncture treatment. One sham acupuncture participant has been mis-labelled as belonging to the real acupuncture group. This error would have had very little impact on the outcomes.

4.6 Correlations

4.6.1 Correlations between objective measures of nasal obstruction

Spearman's rho test revealed a significant negative correlation between PNIF and mean inferior turbinate obstruction only at Week 12 (correlation coefficient = -0.412; p = 0.001**), but not at Week 0 or Week 9. However when all recruited subjects at Week 0 were included in the analysis (n = 149) as opposed to only those who completed the study (n = 110), a significant negative correlation was seen (correlation coefficient = -0.220; p = 0.004*). Although it might be expected intuitively that there would be a clear negative correlation between nasal inspiratory airflow and inferior turbinate obstruction (the more nasal obstruction, the lower the nasal airflow), PNIF is also influenced by lower airways patency (Nathan et al., 2005). It is unclear in this study why there was a significant negative correlation between PNIF and inferior turbinate obstruction at Week 0 (but only when all recruited subjects were included) at Week 12 in the completed subjects, but not at Week 9.

4.6.2 Correlations between subjective measures of nasal obstruction

Spearman's rho test revealed a significant positive correlation between MiniRQLQ nasal symptom domain scores and instantaneous total nasal symptom scores (iTNSS) at Week 0 (correlation coefficient = 0.484; p = 0.001**), at Week 9 (correlation coefficient = 0.541; p = 0.001**) and at Week 12 (correlation coefficient = 0.484; p = 0.001**) (see Table 27). Although these two questionnaires do not measure exactly the same parameters, there is overlap between 3 of the 4 items in the MiniRQLQ nasal symptom score domain and 3 of the 4 symptoms in the iTNSS, namely sneezing, runny nose and stuffy/blocked nose. (The only symptom included in the iTNSS but not the MinRQLQ nasal symptom domain score is itchy nose).

One notable difference between the outcomes produced by the iTNSS and the MiniRQLQ nasal symptom domain score can be seen in the time-point comparisons. The MiniRQLQ showed significant improvements in nasal symptoms from baseline at Week 9 and Week 12 in both the real acupuncture and sham acupuncture groups (see Table 15). However the iTNSS showed significant improvements in nasal symptoms from baseline at Week 9 and Week 12 only in the real acupuncture group and not in the sham group (see Table 12).

Table 27. Correlations between subjective measures of nasal obstruction

			Week 0		Week 9		Week 12	
			iTNSS	MRQLQ nasal	iTNSS	MRQLQ nasal	iTNSS	MRQLQ nasal
Wk 0	iTNSS	cc		**0.484****				
		p value		**p=0.001**				
	MRQLQ nasal	cc	**0.484****					
		p value	**p=0.001**					
Wk 9	iTNSS	cc				**0.541****		
		p value				**p=0.001**		
	MRQLQ nasal	cc			**0.541****			
		p value			**p=0.001**			
Wk 12	iTNSS	cc						**0.484****
		p value						**p=0.001**
	MRQLQ nasal	cc					**0.484****	
		p value					**p=0.001**	

Abbreviations: iTNSS – instantaneous total nasal symptom score, MRQLQ nasal – Mini Rhinoconjunctivitis Quality of Life Questionnaire nasal symptom domain score, cc – correlation coefficient, p value – p value (2-tailed)

* $p < 0.05$ compared to Week 0, ** $p<0.001$ compared to Week 0

4.6.3 Correlations between objective and subjective measures of nasal obstruction

There was no correlation between the objective measures of nasal patency (PNIF and inferior turbinate obstruction) and the subjective measures of perceived nasal obstruction (iTNSS and MiniRQLQ stuffy nose/blocked nose symptom score) at any time-point (see Table 32). Lack of correlation between objective and subjective measures of nasal obstruction has been previously reported and discussed (Baraniuk, 2011; Nathan et al., 2005). A complex interaction between psychological, neurological and mucosal factors appears to be involved in subjective sensations of nasal obstruction (Baraniuk, 2011). For this reason it is valuable to include both objective and subjective measures of nasal obstruction in studies of allergic rhinitis as they provide different information.

4.6.4 Correlations between daily symptom diary and daily medication diary scores

Spearman's rho tests revealed a significant positive correlation between daily symptom diary and daily medication diary mean weekly scores only at Week 10 (correlation coefficient = 0.396; p = 0.028*) in the sham acupuncture group, and at Week 5 (correlation coefficient = 0.440; p = 0.015*) and Week 6 (correlation coefficient = 0.393; p = 0.032*) in the real acupuncture group. This suggests that medication use, as a behaviour, was influenced by factors other than the perceived severity of symptoms. Reduction in the use of relief medications has previously been used in allergic rhinitis studies as a measure of clinical improvement, however it may not be a reliable outcome

measure, unless it is placed in context with other subjective and objective measures of clinical status (Brinkhaus et al., 2013; Xue et al., 2007).

4.7 Responder rate

Some researchers have suggested that acupuncture is only effective for some people and that this group of "responders" is 70% of the population (Longhurst, 2012). To investigate whether there were non-responders in this study, individual subject data for the real acupuncture group was examined. When symptoms and quality of life improved (by at least 10%) on two out of three of the questionnaires used, subjects were deemed to be "responders", while improvement on zero out of three questionnaires constituted "non-responder" status. For those who showed improvement on only one of three questionnaires, a secondary analysis was undertaken of other measures - immunoglobulins, neurotrophins, neuropeptides and nasal patency (a total of 9 measures). When 5 or more out of the 9 measures showed an improvement of at least 10%, the subjects were deemed to be "responders", but when 4 or less out of the 9 measures showed improvement they were regarded as "non-responders". In this study 31 out of 36 subjects (86.1%) were responders while 5 (13.9%) were non-responders.

4.8 Discussion

4.8.1 Nasal patency measures (PNIF and mean inferior turbinate obstruction)

Objective measurement of nasal patency in clinical studies is generally performed using one or more of the following methods: acoustic rhinometry, peak nasal inspiratory flow, peak nasal expiratory flow and rhinoscopic examination to determine nasal obstruction due to swelling at the level of the inferior turbinates (Baraniuk, 2011; Nathan et al., 2005). Peak nasal inspiratory flow is considered more reliable than peak nasal expiratory flow to measure nasal patency, and is inexpensive and simple to administer (Nathan et al., 2005).

In this study, real acupuncture had no impact on objective measures of nasal patency (PNIF and mean inferior turbinate obstruction). Although a time-point difference was

seen between Week 0 and Week 12 in the no acupuncture group on PNIF and a difference in mean inferior turbinate obstruction between the no acupuncture group and the sham acupuncture group in Week 9, as none of the subjects involved received real acupuncture, these differences were unlikely to be related to the effects of acupuncture.

A significant negative correlation was seen between PNIF and mean inferior turbinate obstruction at Week 0 and Week 12 but not at Week 9. It is not known why there was no significant negative correlation at Week 9. It may have been due to factors which influence nasal inspiratory air flow, other than the diameter of the nasal airway at the level of the inferior turbinates, such as lower airways restriction. To reduce such confounding variables, the study design excluded adults who had been diagnosed with asthma, or had been prescribed medication for asthma. During the course of the study two individuals were excluded from the study after commencing medications for asthma. It is possible that lower airways restriction may have played a role in modifying PNIF measurements at Week 9.

4.8.2 Symptom and quality of life scores

According to the daily symptom diary records, significant improvement was first seen in the third week of treatment and persisted until four weeks after the last treatment. All three subjective measures (iTNSS, MiniRQLQ and daily symptom diary) showed significant improvements in symptoms and/or quality of life in the real acupuncture group. In the sham acupuncture group, there were significant improvements shown on the MiniRQLQ, but these improvements were not seen on the iTNSS and the daily symptom diary. On the MiniRQLQ total scores and also on all five MiniRQLQ domain scores there was a consistent pattern of continuing improvement from Week 9 to Week 12 in the real acupuncture group, but of deterioration from Week 9 to Week 12 in the sham acupuncture group.

To further investigate the discrepancies in outcomes between the MiniRQLQ and the iTNSS, a correlation analysis was undertaken to compare the nasal symptom domain score from the MiniRQLQ with the iTNSS score. Spearman's rho test revealed a significant positive correlation between MiniRQLQ nasal symptom domain scores and iTNSS at Week 0 (correlation coefficient = 0.484; p = 0.001**), at Week 9 (correlation coefficient = 0.541; p = 0.001**) and at Week 12 (correlation coefficient = 0.484; p =

0.001**). Although these two questionnaires do not measure exactly the same parameters, there is overlap between 3 of the 4 items in the MiniRQLQ nasal symptom score domain and 3 of the 4 symptoms in the iTNSS, namely sneezing, runny nose and stuffy/blocked nose. The only symptom included in the iTNSS but not the MinRQLQ nasal symptom domain score is itchy nose (see Table 28).

Table 28. Comparison of test items used in three different questionnaires

MiniRQLQ	iTNSS	Daily symptom diary
home and work activities		
recreational activities		
sleep		unrefreshed sleep
need to rub nose/eyes		
need to blow nose		
itchy eyes		eye itch
sore eyes		
watery eyes		
sneezing	sneezing	sneezing
runny nose	runny nose	runny nose
stuffy/blocked nose	blocked nose	
	itchy nose	nasal itch
tiredness and/or fatigue		
thirst		
feeling irritable		
		post nasal drip
		sinus pain

Abbreviations: MiniRQLQ – Mini Rhinoconjunctivitis Quality of Life Questionnaire,
iTNSS - instantaneous total nasal symptom score

4.8.3 Clinical significance of symptom and quality of life scores

To investigate whether or not statistically significant differences were also clinically significant, percentage difference figures were calculated for each of the three questionnaires.

Table 29. Real acupuncture group iTNSS differences at Week 9 and Week 12 compared with Week 0

iTNSS scores	Mean±SE Wk 0	Mean difference±SE Wk 9	% difference Wk 9	Mean difference±SE Wk 12	% difference Wk 12
iTNSS scores	5.42±0.5	1.53±0.6	28.2%	1.86±0.6	34.3%

Abbreviations:iTNSS – instantaneous total nasal symptom score, SE– standard error

Since the improvement in iTNSSscores in the real acupuncture group was 28.2% at Week 9 and 34.3% at Week 12, it is likely that these differences may have been clinically significant (see Table 29).

As the improvements in MiniRQLQ scores in the real acupuncture group (except for "Other symptoms" total score which was not statistically significant) exceeded 20% at Week 9 and exceeded 30% at Week 12, it is likely that these differences may have also been clinically significant (see Table 30).

Table 30. Real acupuncture group MiniRQLQ total and domain score differences at Week 9 and Week 12 compared with Week 0

MiniRQLQ scores	Mean±SE Wk 0	Mean difference±SE Wk 9	% difference Wk 9	Mean difference±SE Wk 12	% difference Wk 12
Total MiniRQLQ scores	44.33±2.7	10.28±2.8	23.2%	13.61±2.9	30.7%
Activities total score	8.67±0.6	1.94±0.6	22.0%	2.56±0.6	30.0%
Practical problems total score	7.89±0.5	2.06±0.5	26.1%	2.72±0.6	34.5%
Nose symptoms total score	11.25±0.7	2.78±0.8	24.7%	3.64±0.8	32.4%
Eye symptoms total score	8.67±0.7	2.50±0.7	28.8%	2.72±0.8	31.4%
Other symptoms total score	7.86±0.7	1.00±0.6	12.7%	1.97±0.6	25.1%

Abbreviations: MiniRQLQ – Mini-Rhinoconjunctivitis Quality of Life Questionnaire, SE – standard error

Table 31. Real acupuncture group daily symptom diary weekly total symptom scores: differences at Week 2 to Week 12 compared with Week 1

Daily symptom diary weekly total symptom scores	Mean±SE	Mean difference±SE compared with Week 1	% difference from Week 1
Week 1	23.3±2.7		
Week 2	21.7±2.9	1.5±1.0	6.6%
Week 3	20.3±3.0	2.9±0.5	12.6%
Week 4	19.0±3.1	4.3±0.8	18.5%
Week 5	18.1±3.2	5.1±0.7	22.0%
Week 6	17.6±3.1	5.6±0.6	24.2%
Week 7	18.7±3.1	4.6±1.4	19.6%
Week 8	18.1±3.1	5.2±1.5	22.3%
Week 9	18.6±3.2	4.6±1.7	19.9%
Week 10	18.5±3.2	4.8±1.8	20.5%
Week 11	18.1±3.2	5.2±1.6	22.2%
Week 12	17.6±3.4	5.7±1.1	24.3%

Abbreviations:iTNSS – instantaneous total nasal symptom score, SE – standard error

4.8.4 Daily medication use diary

The use of relief medication was significantly lower at four weeks follow-up (Week 12) than at baseline, but only in the real acupuncture group. The lack of correlation between daily medication use scores and daily symptoms scores suggests that medication use was influenced by factors other than symptom severity.

4.9 Conclusions

Acupuncture is a safe and effective treatment for adults with persistent allergic rhinitis. Although no changes were seen after acupuncture in objective measurements of nasal patency, there were significant improvements in clinical symptoms, quality of life and use of relief medications. Improvements in symptoms (as shown by the daily symptom diary) first appeared in the third week of treatment and persisted at four-week follow-up.

Different tools to measure outcomes produced different results. PNIF showed a negative correlation with inferior turbinate obstruction at baseline (but only when data from all recruited subjects were included), and at four-week follow-up, but not at one-week follow-up. While iTNSS showed a significant positive correlation with MiniRQLQ at all three time-points measured, the results for the sham acupuncture group showed no improvement when measured with iTNSS, but significant improvement when MiniRQLQ was used. Daily symptom dairy scores also showed no improvement in

symptoms in the sham acupuncture group. This highlights the value of using both subjective and objective outcome measurements, and using multiple outcome measurements in clinical studies of allergic rhinitis.

A significant negative correlation was observed between PNIF and mean inferior turbinate obstruction at Weeks 0 and 12 but not at Week 9. This may have been due to factors other than nasal patency which influence PNIF measurement, such as the status of the lower airways. Objective measurements of nasal patency did not correlate with subjective reports of perceived nasal obstruction. This is consistent with previous studies (Baraniuk, 2011). Subjective sensations of nasal obstruction are influenced by a number of factors apart from nasal patency including neurological, psychological and mucosal factors (Baraniuk, 2011).

The lack of correlation between medication use and symptom severity suggests that there may have been other factors impacting on this behaviour such as psychological factors, or perhaps a sense of medication use being prophylactic rather than responsive to symptoms.

The responder rate to acupuncture treatment was 86.1% in the real acupuncture group in this study.

The sham acupuncture protocol used in this study was shown to be credible to subjects, but not inert. This introduces a negative bias into the study when attempts are made to interpret comparisons between the effects of real and sham acupuncture. Placebo and/or nocebo responses cannot adequately account for the effects of sham acupuncture in this study as the exit debrief questionnaire indicated that expectation and belief were almost identically distributed between the real and sham acupuncture groups.

Table 32. Correlations between objective clinical measurements and subjective symptom and quality of life questionnaires

			Week 0				Week 9				Week 12			
			MITO	PNIF	iTNSS	MRQLQ stuffy	MITO	PNIF	iTNSS	MRQLQ stuffy	MITO	PNIF	iTNSS	MRQLQ stuffy
Wk 0	MITO	cc		-0.137	-0.007	-0.001								
		p		p=0.165	p=0.944	p=0.992								
	PNIF	cc	-0.137		-0.046	-0.006								
		p	p=0.165		p=0.521	p=0.950								
	iTNSS	cc	-0.007	-0.046		**0.512****								
		p	p=0.944	p=0.521		**p=0.001**								
	MRQLQ stuffy	cc	-0.001	-0.006	**0.512****									
		p	p=0.992	p=0.950	**p=0.001**									
Wk 9	MITO	cc						-0.114	-0.074	0.009				
		p						P=0.278	P=0.451	P=0.928				
	PNIF	cc					-0.114		-0.026	-0.004				
		p					P=0.278		P=0.803	P=0.972				
	iTNSS	cc					-0.074	-0.026		**0.565****				
		p					P=0.451	P=0.803		**p=0.001**				
	MRQLQ stuffy	cc					0.009	0.004	**0.565****					
		p					P=0.928	P=0.972	**p=0.001**					
Wk 12	MITO	cc										-**0.412****	0.127	0.090
		p										**p=0.001**	P=0.196	P=0.361
	PNIF	cc									**-0.412****		-0.133	0.030
		p									**p=0.001**		P=0.224	P=0.787
	iTNSS	cc									0.127	-0.133		**0.439****
		p									P=0.196	P=0.224		**p=0.001**
	MRQLQ stuffy	cc									0.090	0.030	**0.439****	
		p									P=0.361	P=0.787	**p=0.001**	

Abbreviations: MITO – mean inferior turbinate obstruction, PNIF – peak nasal inspiratory flow, iTNSS – instantaneous total nasal symptom score, MRQLQ stuffy – Mini Rhinoconjunctivitis Quality of Life Questionnaire stuffy nose/blocked nose score, cc – correlation coefficient, p value – p value (2-tailed)

**p< 0.001

Chapter 5. Laboratory analysis

5.1 Abstract

Background: Laboratory testing of biomarkers in blood serum, blood plasma and saliva samples was undertaken to determine whether any modulation in mucosal immunity had occurred after acupuncture in adults with persistent allergic rhinitis.

Objectives: Four main categories of biomarkers were investigated – immunoglobulin E and allergen-specific IgE antibodies, neurotrophins, pro-inflammatory neuropeptides and cytokines. Down-regulation of total IgE, allergen-specific IgE antibodies, neurotrophins and pro-inflammatory neuropeptides and a shift in Th1/Th2 helper cell ratio away from Th2 dominance following acupuncture, would indicate a down-regulation of the allergic inflammatory response. Another aim of this study was to compare any changes in biomarker data with changes in nasal airway resistance, symptoms and quality of life, to provide a better understanding of the effects of acupuncture in allergic rhinitis.

Methods: Methods are described in Chapter 2.

Results: A significant reduction was found in total IgE and allergen-specific IgE for house dust mite after acupuncture, but not in allergen-specific IgE for three regional grass pollens. No significant changes were seen in the neurotrophins. The pro-inflammatory neuropeptide SP was significantly reduced after acupuncture in Week 1 and Week 3 but SP levels had begun to rise again by four-week follow-up. VIP was significantly reduced in the real acupuncture group compared to the sham acupuncture group at 18 to 24 hours post treatment in Week 1, however there were no significant differences between time-points within any group. CGRP did not change significantly. There were no significant changes in CGRP, cytokines or ECP between Week 0 and Week 12.

Conclusions: Previous studies have reported down-regulation of total IgE, SP and VIP in allergic rhinitis sufferers following acupuncture. In this study total IgE and specific IgE for house dust mite both decreased significantly. Down-regulation of SP and VIP

was seen but this effect was short-lived. There were no significant changes in NGF, BDNF, CGRP, cytokines or ECP at four-week follow-up.

5.2 Introduction

The primary outcome for the study was to measure any potential modulation of the mucosal immune response in the upper respiratory tract by acupuncture in subjects with allergic rhinitis. The secondary outcome was to measure any potential reduction in markers of mucosal inflammation (nasal airway resistance, symptoms and eosinophilic cationic protein) and improved quality of life scores following acupuncture.

Measurements of nasal airway resistance, symptoms and quality of life have been detailed in Chapter 4. In this chapter the laboratory analysis of four main categories of biomarkers (immunoglobulin E, neurotrophins, pro-inflammatory neuropeptides and cytokines) as well as the inflammatory marker, ECP, will be examined for any evidence of modulation of the mucosal immune response in the upper respiratory tract. Any modulation of the mucosal immune response detected by analysis of the biomarkers could then be compared with any improvements which occurred in nasal airway resistance, symptoms and quality of life.

5.3 Methods

Methods are described in Chapter 2.

5.4 Results

5.4.1 Total and allergen-specific immunoglobulin E (IgE)

None of the values for total or allergen-specific IgE were found to be normally distributed on Kolgomorov-Smirnov and Shapiro-Wilk tests for normality, however logged values for total IgE and allergen-specific IgE for house dust mite were normal. Hence comparisons between Week 0 and Week 12 for each group were tested with paired-samples T tests with logged data for total IgE and house dust mite specific IgE. For the three grass pollen specific IgEs, Friedman's test was used. Between-group comparisons for total IgE and specific IgE for house dust mite were performed with

Independent-samples T test while the allergen-specific IgE antibodies for the three grass pollens were tested with the Independent-samples Kruskall-Wallis test.

5.4.1.1 Total IgE

5.4.1.1.1 Time-point comparisons within groups

Total IgE in plasma was compared between baseline (Week 0) and four-week follow-up (Week 12) for each group. There were significant differences between Week 0 and Week 12 in the real acupuncture group (p = 0.016*) and the sham acupuncture group (p = 0.015*), but no significant difference in the no acupuncture group (p = 0.216) (see Table 1).

Table 1. Logged total IgE in serum (kU/L): Time-point comparisons within groups

	Week	Mean (±SE)	Mean Difference (±SE)	P value (2-tailed)
No acupuncture (n = 34)	0	2.19±0.09	0.02±0.02	0.216
	12	2.17±0.08		
Sham acupuncture (n = 35)	0	2.17±0.09	0.04±0.01	0.015*
	12	2.13±0.09		
Real acupuncture (n = 32)	0	2.03±0.01	0.04±0.01	0.016*
	12	1.99±0.02		

Abbreviations: IgE – immunoglobulin E, n – number of subjects, SE – standard error, kU/L – kilounits per litre

* p < 0.05 compared to Week 0

5.4.1.1.2 Time-point comparisons between groups

Between-group comparisons for each week were performed with the Independent-Samples T test. There were no significant differences between groups at Week 0 or Week 12 (see Table 2).

Table 2. Logged total IgE in serum (kU/L): Time-point comparisons between groups

	Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Week	Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0	-0.12(-0.37 to 0.12)	0.309	-0.11(-0.36 to 0.14)	0.379	-0.01(-0.26 to 0.24)	0.916
12	-0.17(-0.40 to 0.06)	0.145	-0.15(-0.39 to 0.10)	0.232	-0.02(-0.27 to 0.22)	0.850

Abbreviations: kU/L – kilounits per litre, 95% CI – 95% confidence interval

5.4.1.2 Allergen-specific IgE for Bahia grass

5.4.1.2.1 Time-point comparisons within groups

Allergen-specific IgE in plasma for Bahia grass was compared between Week 0 and Week 12 for each group. There was a significant difference between Week 0 and Week 12 in the no acupuncture group (p = 0.012*) but no significant differences in the sham acupuncture group (p = 0.285) or real acupuncture group (p = 0.819) [Friedman's test] (see Table 3).

Table 3. Allergen-specific IgE for Bahia grass in serum (kU/L): Time-point comparisons within groups:

Group	Test statistic	Degrees of freedom	P value (2-tailed)
No acupuncture (n = 35)	6.250	1	p = 0.012*
Sham acupuncture (n = 35)	1.143	1	p = 0.285
Real acupuncture (n = 32)	0.053	1	p = 0.819

Abbreviations: IgE – immunoglobulin E, kU/L – kilounits per litre, n – number of subjects

* p < 0.05 compared to Week 0

5.4.1.2.2 Time-point comparisons between groups

Between-group comparisons for each week were performed with the Independent-Samples Kruskal-Wallis test. There were no significant differences between groups at Week 0 or Week 12 (see Table 4).

Table 4. Allergen-specific IgE for Bahia grass in serum (kU/L): Time-point comparisons between groups

Week	P value (2-tailed)	n	Test statistic	Degrees of freedom
0	0.251	106	2.764	2
12	0.306	102	2.370	2

Abbreviations: kU/L – kilounits per litre, n – number of subjects

5.4.1.3 Allergen-specific IgE for Johnson grass

5.4.1.3.1 Time-point comparisons within groups

Allergen-specific IgE in plasma for Johnson grass was compared between Week 0 and Week 12. There were no significant differences between Week 0 and Week 12 in any group [Friedman's test] (see Table 5).

Table 5. Allergen-specific IgE for Johnson grass in serum (kU/L): Time-point comparisons within groups

Group	Test statistic	Degrees of freedom	P value (2-tailed)
No acupuncture (n = 35)	0.067	1	p = 0.796
Sham acupuncture (n = 35)	0.286	1	p = 0.593
Real acupuncture (n = 32)	1.471	1	p = 0.225

Abbreviations: IgE – immunoglobulin E, kU/L – kilounits per litre, n – number of subjects

5.4.1.3.2 Time-point comparisons between groups

Between-group comparisons for each week were performed with the Independent-Samples Kruskal-Wallis test. There were no significant differences between groups at Week 0 or Week 12 (see Table 6).

Table 6. Allergen-specific IgE for Johnson grass in serum (kU/L): Time-point comparisons between groups

Week	P value (2-tailed)	n	Test statistic	Degrees of freedom
0	0.310	106	2.345	2
12	0.329	102	2.221	2

Abbreviations: kU/L – kilounits per litre, n – number of subjects

5.4.1.4 Allergen-specific IgE for Bermuda grass

5.4.1.4.1 Time-point comparisons within groups

Allergen-specific IgE in plasma for Bermuda grass was compared between Week 0 and Week 12 for each group. There were no significant differences between Week 0 and Week 12 in any group [Friedman's test] (see Table 7).

Table 7. Allergen-specific IgE for Bermuda grass in serum (kU/L): time-point comparisons within groups

Group	Test statistic	Degrees of freedom	P value (2-tailed)
No acupuncture (n = 35)	2.882	1	p = 0.090
Sham acupuncture (n = 36)	0.600	1	p = 0.439
Real acupuncture (n = 33)	0.059	1	p = 0.808

Abbreviations: IgE – immunoglobulin E, kU/L – kilounits per litre, n – number of subjects

5.4.1.4.2 Time-point comparisons between groups

Between-group comparisons for each week were performed with the Independent-Samples Kruskal-Wallis test. There were no significant differences between groups at Week 0 or Week 12 (see Table 8).

Table 8. Allergen-specific IgE for Bermuda grass in serum (kU/L): Time-point comparisons between groups

Week	P value (2-tailed)	n	Test statistic	Degrees of freedom
0	0.494	106	1.410	2
12	0.415	104	1.758	2

Abbreviations: kU/L – kilounits per litre, n – number of subjects

5.4.1.5 Allergen-specific IgE for house dust mite

5.4.1.5.1 Time-point comparisons within groups

Allergen-specific IgE in plasma for house dust mite was compared between Week 0 and Week 12 for each group. There was a significant difference between Week 0 and Week 12 in the real acupuncture group (p = 0.035*) but no significant differences in the sham acupuncture group (p = 0.979) or the no acupuncture group (p = 0.796) (see Table 9).

Table 9. Logged specific IgE for house dust mite in serum (kU/L): time-point comparisons within groups

	Week	Mean (±SE)	Mean Difference (±SE)	P value (2-tailed)
No acupuncture (n = 34)	0	1.07±0.12	0.01±0.02	0.796
	12	1.07±0.13		
Sham acupuncture (n = 35)	0	1.11±0.13	0.00±0.02	0.979
	12	1.11±0.13		
Real acupuncture (n = 32)	0	0.81±0.14	0.04±0.02	0.035*
	12	0.77±0.14		

Abbreviations: IgE – immunoglobulin E, kU/L – kilounits per litre, n – number of subjects, SE – standard error

* $p < 0.05$ compared to Week 0

5.4.1.5.2 Time-point comparisons between groups

Between-group comparisons for each week were performed with the Independent-Samples Kruskal-Wallis test. There were no significant differences between groups at Week 0 or Week 12 (see Table 10).

Table 10. Logged specific IgE for house dust mite in serum (kU/L): time-point comparisons between groups

Week	Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
	Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0	-0.17(-0.53 to 0.20)	0.373	-0.19(-0.57 to 0.19)	0.311	0.03(-0.33 to 0.39)	0.877
12	-0.32(-0.68 to 0.04)	0.081	-0.31(-0.68 to 0.06)	0.097	-0.01(-0.36 to 0.35)	0.973

Abbreviations: IgE – immunoglobulin E, kU/L – kilounits per litre, 95% CI – 95% confidence interval

5.4.2 Neurotrophins

5.4.2.1 Nerve growth factor (NGF)

5.4.2.1.1 Time-point comparisons within groups

Levels of NGF in plasma were compared between baseline (Week 0) and four-week follow-up (Week 12) for each group. Since Kolmogorov-Smirnov and Shapiro-Wilk tests for normality indicated that NGF was not normally distributed in any group, data was log-transformed (after which it was normal) then analysed with Paired T tests. There were no significant differences between Week 0 and Week 12 in any group (see Table 11).

Table 11. Logged NGF in plasma (pg/ml): time-point comparisons within groups

	Week	Mean (±SE)	Mean Difference (±SE)	P value (2-tailed)
No acupuncture (n = 33)	0	0.41±0.08	-0.01±0.05	0.861
	12	0.42±0.08		
Sham acupuncture (n = 34)	0	0.26±0.08	-0.05±0.04	0.217
	12	0.32±0.08		
Real acupuncture (n = 32)	0	0.30±0.08	-0.04±0.03	0.219
	12	0.34±0.08		

Abbreviations: NGF – nerve growth factor, pg/ml – picograms per millilitre, n – number of subjects, SE – standard error

5.4.2.1.2 Time-point comparisons between groups

Independent samples T tests with logged data were used to compare groups at Week 0 and Week 12. There were no significant differences between groups at either Week 0 or Week 12 (see Table 12).

Table 12. Logged NGF in plasma (pg/ml): time-point: comparisons between groups

	Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Week	Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0	-0.15(-0.37 to 0.07)	0.190	0.04(-0.18 to 0.26)	0.708	-0.19(-0.41 to 0.03)	0.094
12	-0.01(-0.22 to 0.21)	0.947	0.09(-0.13 to 0.30)	0.420	-0.09(-0.31 to 0.12)	0.390

Abbreviations: NGF – nerve growth factor, pg/ml – picograms per millilitre, 95% CI – 95% confidence interval

5.4.2.2 Brain-derived neurotrophic factor (BDNF)

5.4.2.2.1 Time-point comparisons within groups

Levels of BDNF in plasma were compared between Week 0 and Week 12 for each group. Since Kolmogorov-Smirnov and Shapiro-Wilk tests for normality indicated that BDNF was not normally distributed, data was log-transformed then analysed with Paired T tests. There were no significant differences between Week 0 and Week 12 in any group (see Table 13).

Table 13. Logged BDNF in plasma (pg/ml): time-point comparisons within groups

	Week	Mean (±SE)	Mean Difference (±SE)	P value (2-tailed)
No acupuncture (n = 33)	0	3.19±0.06	0.00±0.05	0.960
	12	3.19±0.06		
Sham acupuncture (n = 34)	0	3.22±0.05	-0.06±0.05	0.224
	12	3.27±0.05		
Real acupuncture (n = 33)	0	3.23±0.05	-0.06±0.03	0.082
	12	3.30±0.06		

Abbreviations: BDNF – brain-derived neurotrophic factor, pg/ml – picograms per millilitre, n – number of subjects, SE – standard error

5.4.2.2.2 Time-point comparisons between groups

Independent-samples T tests with logged data were used to compare groups at Week 0 and Week 12. There were no significant differences between groups at either Week 0 or Week 12 (see Table 14).

Table 14. Logged BDNF in plasma (pg/ml): time-point comparisons between groups

	Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Week	Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0	0.04(-0.11 to 0.18)	0.631	0.02(-0.12 to 0.16)	0.769	0.02(-0.14 to 0.17)	0.848
12	0.09(-0.07 to 0.26)	0.253	0.01(-0.13 to 0.16)	0.578	-0.09(-0.31to 0.12)	0.318

Abbreviations: BDNF – brain-derived neurotrophic factor, pg/ml – picograms per millilitre , 95% CI – 95% confidence interval

5.4.3 Pro-inflammatory Neuropeptides

Levels of three pro-inflammatory neuropeptides were measured – Substance P (SP), vaso-active intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP). Initially all three neuropeptides were measured in saliva however levels of CGRP were below detectable threshold in saliva, so CGRP was subsequently measured in plasma.

5.4.3.1 Substance P (SP)

5.4.3.1.1 Time-point comparisons within groups

Levels of SP in saliva were compared between Weeks 1, 3, 6 and 12 for each group. In the real and sham acupuncture groups, samples were collected pre-treatment in Week 1 and 18 to 24 hours post-treatment in Weeks 1, 3 and 6. In the no acupuncture group, the collection of post-treatment samples was not possible, however samples were collected in Weeks 1, 3 and 6. Samples were also collected from all groups in Week 12 at the allergy clinic.

Since Kolmogorov-Smirnov and Shapiro-Wilk tests for normality indicated that SP was not normally distributed in any group, data was log-transformed (after which it was normal) then analysed with Paired T tests. In the real acupuncture group there were significant decreases in SP between Week 1 pre-treatment and 18 to 24 hours post-treatment in both Week 1 ($p = 0.039^*$) and Week 3 ($p = 0.041^*$) (see Table 15). In the sham acupuncture group there were significant decrease in SP between Week 1 pre-treatment and Week 6 (18 to 24 hours post-treatment) ($p = 0.027^*$) There were no significant differences between time-points in the no acupuncture group (see Table 15).

5.4.3.1.2 Time-point comparisons between groups

Independent samples T tests (with log-transformed data) were used to compare real and sham acupuncture groups at each time-point. There were no significant differences between real and sham acupuncture groups at any time-point (see Table 16). To compare the no acupuncture group with the other two groups, since the time-points were not identical, similar time-points were used (see Table 17). There were significant differences between the real acupuncture and no acupuncture groups in Week 3 (p = 0.002*) and Week 6 (p = 0.015*) and also between the sham acupuncture and no acupuncture groups in Week 3 (p = 0.027*) and Week 6 (p = 0.003*) (see Table 17).

Table 15. Logged SP in saliva (pg/ml): time-point comparisons within groups

	n	Week	Mean (±SE)	Mean Difference (±SE) from Week 1 pre	P value (2-tailed)
No acupuncture	37	1	2.05±0.15		
	37	3	2.02±0.14	0.03±0.08	0.693
	37	6	2.02±0.14	0.03±0.67	0.485
	37	12	1.95±0.15	0.06±0.54	0.419
Sham acupuncture	36	1 pre	1.69±0.14		
	36	1 (18-24 hrs)	1.40±0.16	0.29±0.17	0.103
	33	3 (18-24 hrs)	1.53±0.18	0.10±0.16	0.523
	34	6 (18-24 hrs)	1.41±0.16	0.33±0.14	0.027*
	36	12	1.76±0.17	-0.07±0.16	0.676
Real acupuncture	36	1 pre	1.75±0.14		
	34	1 (18-24 hrs)	1.57±0.12	0.24±0.11	0.039*
	33	3 (18-24 hrs)	1.40±0.14	0.34±0.16	0.041*
	33	6 (18-24 hrs)	1.57±0.13	0.20±0.18	0.256
	36	12	1.64±0.14	0.11±0.16	0.475

Abbreviations: Logged SP – log-transformed values to base 10 for Substance P, pg/ml – picograms per millilitre, n – number of subjects, SE – standard error, pre – pre-treatment, 18-24 hrs – 18 to 24 hours post-treatment

***** p < 0.05 compared to Week 1 pre-treatment

Table 16. Logged SP in saliva (pg/ml): comparisons between groups at same time-points

Week	Real acupuncture vs sham acupuncture	
	Difference (95% CI)	P value
1 pre-treatment	-0.06 (-0.46 to 0.33)	0.761
1 (18-24 hrs post)	-0.16 (-0.58 to 0.24)	0.416
3 (18-24 hrs post)	0.14 (-0.31 to 0.59)	0.545
6 (18-24 hrs post)	-0.17 (-0.58 to 0.25)	0.427
12	0.12 (-0.32 to 0.56)	0.581

Abbreviations: Logged SP – log-transformed values to base 10 of Substance P, pg/ml – picograms per millilitre, 95 % CI – 95% confidence interval

Table 17. Logged SP in saliva (pg/ml): comparisons between groups at similar time-points

	Real acupuncture vs no acupuncture		Sham acupuncture vs no acupuncture	
Week	Difference (95% CI)	P value	Difference (95% CI)	P value
Wk 1 pre vs Wk 1	-0.28 (-0.68 to 0.12)	0.164	-0.34 (-0.74 to 0.06)	0.096
Wk 3 (18-24) vs Wk 3	-0.64 (-1.03 to -0.25)	0.002*	-0.51 (-0.95 to -0.06)	0.027*
Wk 6 (18-24) vs Wk 6	-0.49 (-0.88 to -0.10)	0.015*	-0.66 (-1.08 to -0.23)	0.003*
12	-0.33 (-0.74 to 0.07)	0.107	-0.21 (-0.65 to 0.23)	0.348

Abbreviations: Logged SP – log-transformed values to base 10 for Substance P, pg/ml – picograms per millilitre, 95 % CI – 95% confidence interval, pre – pre-treatment, 18-24 hrs – 18 to 24 hours post-treatment

* $p < 0.05$ compared to Week 1 pre-treatment/Week 1

5.4.3.2 Vaso-active intestinal peptide (VIP)

5.4.3.2.1 Time-point comparisons within groups

Levels of VIP in saliva were compared between Weeks 1, 3, 6 and 12 for each group. In the real and sham acupuncture groups, samples were collected pre-treatment in Week 1 and 18 to 24 hours post-treatment in Weeks 1,3 and 6. In the no acupuncture group the collection of post-treatment samples was not possible, however samples were collected in Weeks 1,3 and 6. Samples were also collected from all groups in Week 12 at the allergy clinic. Since Kolmogorov-Smirnov and Shapiro-Wilk tests for normality indicated that VIP was not normally distributed in any group, data was log-transformed (after which it was normal) then analysed with Paired T tests. There were no significant differences between Week 1 pre-treatment and any other time-point (see Table 18).

Table 18. Logged VIP in saliva (pg/ml): time-point comparisons within groups

	n	Week	Mean (±SE)	Mean Difference (±SE) from Week 1 pre	P value (2-tailed)
No acupuncture	37	1	1.13±0.11		
	37	3	1.25±0.11	-0.12±0.54	0.195
	37	6	1.25±0.11	-0.14±0.75	0.275
	34	12	1.19±0.08	-0.09±0.65	0.428
Sham acupuncture	36	1 pre	1.23±0.11		
	33	1 (18-24 hrs)	1.38±0.12	-0.11±0.13	0.382
	33	3 (18-24 hrs)	1.25±0.12	-0.04±0.15	0.776
	31	6 (18-24 hrs)	1.21±0.12	0.04±0.14	0.784
	35	12	1.24±0.11	0.02±0.10	0.863
Real acupuncture	36	1 pre	1.07±0.11		
	30	1 (18-24 hrs)	1.07±0.08	-0.02±0.12	0.849
	32	3 (18-24 hrs)	1.16±0.10	-0.03±0.12	0.788
	32	6 (18-24 hrs)	1.13±0.10	-0.04±0.14	0.806
	34	12	1.14±0.09	-0.08±0.13	0.523

Abbreviations: Logged VIP – log-transformed values to base 10 for Vaso-active intestinal peptide, pg/ml – picograms per millilitre, n – number of subjects, SE – standard error, SD – standard deviation, pre – pre-treatment, 18-24 hrs – 18 to 24 hours post-treatment

5.4.3.2.2 Time-point comparisons between groups

Independent samples T tests (with log-transformed data) were used to compare real and sham acupuncture groups at each time-point. VIP levels were significantly lower in the real acupuncture group compared to the sham acupuncture group at Week 1 (18-24 hours post-treatment) ($p = 0.041$*) (see Table 19). To compare the no acupuncture group with the other two groups, since the time-points were not identical, similar time-points were used (see Table 20). There were no significant differences between groups (see Table 20).

Table 19. Logged VIP in saliva (pg/ml): comparisons between groups at same time-points

	Real acupuncture vs sham acupuncture	
Week	Difference (95% CI)	P value
1 pre-treatment	0.16 (-0.41 to 0.46)	0.292
1 (18-24 hrs post)	0.31 (0.01 to 0.60)	0.041*
3 (18-24 hrs post)	0.09 (-0.22 to 0.41)	0.556
6 (18-24 hrs post)	0.08 (-0.25 to 0.40)	0.637
12	0.10 (-0.19 to 0.39)	0.488

Abbreviations: Logged VIP – log-transformed values to base 10 of Substance P, pg/ml – picograms per millilitre, 95% CI – 95% confidence interval

* $p < 0.05$ compared to Week 1 pre-treatment

Table 20. Logged VIP in saliva (pg/ml): comparisons between groups at similar time-points

	Real acupuncture vs no acupuncture		Sham acupuncture vs no acupuncture	
Week	Difference (95% CI)	P value	Difference (95% CI)	P value
Wk 1 pre vs Wk 1	-0.05 (-0.35 to 0.26)	0.762	0.12 (-0.19 to 0.42)	0.454
Wk 3 (18-24) vs Wk 3	-0.09 (-0.40 to 0.21)	0.533	-0.00 (-0.34 to 0.33)	0.996
Wk 6 (18-24) vs Wk 6	-0.11 (-0.42 to 0.20)	0.474	-0.03 (-0.37 to 0.30)	0.837
12	-0.03 (-0.28 to 0.22)	0.810	0.07 (-0.21 to 0.35)	0.619

Abbreviations: Logged VIP – log-transformed values to base 10 for Vaso-active intestinal peptide, pg/ml – picograms per millilitre, 95% CI – 95% confidence interval, pre – pre-treatment, 18-24 hrs – 18 to 24 hours post-treatment

5.4.3.3 Calcitonin gene-related peptide (CGRP)

5.4.3.3.1 Time-point comparisons within groups

CGRP levels in plasma were compared between baseline (Week 0) and four-week follow-up (Week 12) for each group. Original values for CGRP were not normally distributed, however log transformed values were. Hence, paired T tests with log-transformed data were performed. There were no significant differences between Week 0 and Week 12 in any group (See Table 21)

Table 21. Logged CGRP in plasma (pg/ml): time-point comparsions within groups

	Week	Mean (±SE)	Mean Difference (±SE)	P value (2-tailed)
No acupuncture (n = 33)	0	1.29±0.08	-0.07±0.05	0.176
	12	1.36±0.07		
Sham acupuncture (n = 34)	0	1.25±0.08	-0.06±0.08	0.410
	12	1.32±0.08		
Real acupuncture (n = 35)	0	1.29±0.07	- 0.09±0.06	0.128
	12	1.39±0.07		

Abbreviations: CGRP – calcitonin gene-related peptide, pg/ml – picograms per millilitre, n – number of subjects, SE – standard error

5.4.3.3.2 Time-point comparisons between groups

Independent samples T tests with logged data were used to compare groups at Week 0 and Week 12. There were no significant differences between groups at either Week 0 or Week 12 (see Table 22).

Table 22. Logged CGRP in plasma (pg/ml): time-point comparisons between groups

Week	Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
	Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0	0.02(-0.19 to 0.23)	0.843	0.40(-0.18 to 0.25)	0.734	-0.02(-0.23 to 0.20)	0.884
12	0.04(-0.15to 0.23)	0.677	0.09(-0.12 to 0.30)	0.392	-0.05(-0.26 to 0.16)	0.647

Abbreviations: CGRP – calcitonin gene-related peptide, pg/ml – picograms per millilitre, 95% CI – 95% confidence interval

5.4.4 Other inflammatory markers: Eosinophilic cationic protein (ECP)

5.4.4.1 Time-point comparisons within groups

Since values for ECP were not normally distributed on Kolmogorov-Smirnov and Shapiro-Wilk tests for normality, nor were log-transformed values normally distributed, Friedman's test was used to compare baseline (Week 0) ECP levels in saliva with four-week follow-up (Week 12) levels. There were no significant differences in any group (see Table 23).

Table 23. ECP in saliva (ng/ml): time-point comparison within groups

Group	Test statistic	Degrees of freedom	P value (2-tailed)
No acupuncture (n = 32)	0.615	1	0.433
Sham acupuncture (n = 34)	0.037	1	0.847
Real acupuncture (n = 29)	0.001	1	1.000

Abbreviations: ECP – eosinophilic cationic protein, nanograms per millitre, n – number of subjects

5.4.4.2 Time-point comparisons between groups

Between-group comparisons for each week were performed with the Independent-Samples Kruskal-Wallis test. There were no significant differences between groups at Week 0 or Week 12 (see Table 24).

Table 24. ECP in saliva (ng/ml): time-point comparisons between groups

Week	P value (2-tailed)	n	Test statistic	Degrees of freedom
0	0.816	100	0.407	2
12	0.959	102	0.083	2

Abbreviations: ECP – eosinophilic cationic protein, nanograms per millitre, n – number of subjects

5.4.5 Cytokines

The following cytokines were measured in plasma at Week 0 and Week 12: interleukin 2 (IL2), interleukin 4 (IL4), interleukin 10 (IL10), interleukin 12 [p70] (IL12[p70]), interferon gamma (IFNγ) and eotaxin.

5.4.5.1 Interleukin 2 (IL-2)

5.4.5.1.1 Time-point comparisons within groups

IL-2 values were not normally distributed on Kolmogorov-Smirnov and Shapiro Wilk tests for normality, nor were log-transformed values normal. Hence, Friedman's tests were used to compare IL-2 at Week 0 with Week 12 for each group. There were no significant differences in IL-2 in any group (see Table 25).

Table 25. IL- 2 in plasma (pg/ml): time-point comparisons within groups

Group	Test statistic	Degrees of freedom	P value (2-tailed)
No acupuncture (n = 33)	0.154	1	0.695
Sham acupuncture (n = 30)	0.001	1	1.000
Real acupuncture (n = 33)	0.077	1	0.782

Abbreviations: IL- 2 – Interleukin 2, pg/ml – picograms per millilitre, n – number of subjects

5.4.5.1.2 Time-point comparisons between groups

Between-group comparisons for each week were performed with the Independent-Samples Kruskal-Wallis test. There were significant differences between groups at both Week 0 (p = 0.019*) and Week 12 (p = 0.045*) (see Table 26).

Table 26. IL- 2 in plasma (pg/ml): time-point comparisons between groups

Week	P value (2-tailed)	n	Test statistic	Degrees of freedom
0	0.019*	99	7.940	2
12	0.045*	99	6.192	2

Abbreviations: IL- 2 – Interleukin 2, pg/ml – picograms per millilitre, n – number of subjects

5.4.5.2 Interleukin 4 (IL-4)

5.4.5.2.1 Time-point comparisons within groups

IL-4 values were not normally distributed on Kolmogorov-Smirnov and Shapiro Wilk tests for normality, nor were log-transformed values normal. Hence, Friedman's tests were used to compare IL-4 at Week 0 with Week 12 for each group. There were no significant differences in IL-4 in any group (see Table 27).

Table 27. IL- 4 in plasma (pg/ml): time-point comparisons within groups

Group	Test statistic	Degrees of freedom	P value (2-tailed)
No acupuncture (n = 33)	0.360	1	0.549
Sham acupuncture (n = 32)	0.600	1	0.439
Real acupuncture (n = 30)	2.000	1	0.157

Abbreviations: IL- 4 – Interleukin 4, pg/ml – picograms per millilitre, n – number of subjects

5.4.5.2.2 Time-point comparisons between groups

Between-group comparisons for each week were performed with the Independent-Samples Kruskal-Wallis test. There were significant differences between groups at both Week 0 (p = 0.018*) and Week 12 (p = 0.018*) (see Table 28).

Table 28. IL- 4 in plasma (pg/ml): time-point comparisons between groups

Week	P value (2-tailed)	n	Test statistic	Degrees of freedom
0	0.018*	97	8.005	2
12	0.018*	101	8.011	2

Abbreviations: IL- 4 – Interleukin 4, pg/ml – picograms per millilitre, n – number of subjects

* $p < 0.05$ compared to Week 0

5.4.5.3 Interleukin 10 (IL-10)

5.4.5.3.2 Time-point comparisons within groups

IL-10 values were not normally distributed on Kolmogorov-Smirnov and Shapiro Wilk tests for normality, nor were log-transformed values normal. Hence, Friedman's tests were used to compare IL-10 at Week 0 with Week 12 for each group. There were no significant differences in IL-10 in any group (see Table 29)

Table 29. IL- 10 in plasma (pg/ml): time-point comparisons within groups

Group	Test statistic	Degrees of freedom	P value (2-tailed)
No acupuncture (n = 33)	0.926	1	0.336
Sham acupuncture (n = 31)	0.474	1	0.491
Real acupuncture (n = 32)	1.000	1	0.317

Abbreviations: IL-- 10 – Interleukin 10, pg/ml – picograms per millilitre, n – number of subjects

5.4.5.3.2 Time-point comparisons between groups

Between-group comparisons for each week were performed with the Independent-Samples Kruskal-Wallis test. There were significant differences between groups at both Week 0 (p = 0.021*) and Week 12 (p = 0.011*) (see Table 30).

Table 30. IL- 10 in plasma (pg/ml): Time-point comparisons between groups

Week	P value (2-tailed)	n	Test statistic	Degrees of freedom
0	0.021*	99	7.743	2
12	0.011*	101	8.994	2

Abbreviations: IL- 10 – Interleukin 10, pg/ml – picograms per millilitre, n – number of subjects

***** p < 0.05 compared to Week 0

5.4.5.4 Interleukin 12 (p70) [IL-12 (p70)

5.4.5.4.1 Time-point comparisons within groups

IL-12 (p70) values were not normally distributed on Kolmogorov-Smirnov and Shapiro Wilk tests for normality, nor were log-transformed values normal. Hence, Friedman's tests were used to compare IL-12 (p70) at Week 0 with Week 12 for each group. There were no significant differences in IL-12 (p70) in any group (see Table 31).

Table 31. IL- 12(p70) in plasma (pg/ml): time-point comparisons within groups

Group	Test statistic	Degrees of freedom	P value (2-tailed)
No acupuncture (n = 37)	0.471	1	0.493
Sham acupuncture (n = 34)	0.167	1	0.683
Real acupuncture (n = 32)	3.000	1	0.083

Abbreviations: IL- 12(p70) – Interleukin 12(p70), pg/ml – picograms per millilitre, n – number of subjects

5.4.5.4.2 Time-point comparisons between groups

Between-group comparisons for each week were performed with the Independent-Samples Kruskal-Wallis test. There were significant differences between groups at Week 0 (p = 0.022*), but not at Week 12 (p = 0.127) (see Table 32).

Table 32. IL- 12 (p70) in plasma (pg/ml): Time-point comparisons between groups

Week	P value (2-tailed)	n	Test statistic	Degrees of freedom
0	0.022*	104	7.652	2
12	0.127	103	4.120	2

Abbreviations: IL- 12(p70) – Interleukin 12(p70), pg/ml – picograms per millilitre, n – number of subjects

* p < 0.05 compared to Week 0

5.4.5.5 Interferon gamma (IFNγ)

5.4.5.5.1 Time-point comparisons within groups

IFNγ values were not normally distributed on Kolmogorov-Smirnov and Shapiro Wilk tests for normality, however log-transformed values were normal. Hence, paired T tests with log-transformed values were used to compare IFNγ at Week 0 with Week 12 for each group. There were no significant differences in IFNγ in any group (see Table 33).

Table 33. Logged IFNγ in plasma (pg/ml): time-point comparisons within groups

	Week	Mean (±SE)	Mean Difference (±SE)	P value (2-tailed)
No acupuncture (n = 33)	0	1.08±0.13	-0.09±0.09	0.304
	12	1.17±0.13		
Sham acupuncture (n = 34)	0	0.75±0.09	-0.15±0.09	0.118
	12	0.90±0.11		
Real acupuncture (n = 35)	0	1.08±0.13	0.08±0.08	0.402
	12	1.01±0.13		

Abbreviations: Logged IFNγ - Log-transformed value to base 10 of interferon gamma , pg/ml – picograms per millilitre, n – number of subjects, SE – standard error, SD – standard deviation

5.4.5.5.2 Time-point comparisons between groups

Independent samples T tests with logged data were used to compare groups at Week 0 and Week 12. At Week 0 there were significant differences between real and sham acupuncture groups (p = 0.014*) and between sham and no acupuncture groups (p = 0.033*) (see Table 34). Since no changes were observed in IFNγ between time-points in any group, this disparity in groups at baseline does not appear to have had any significant impact on the outcomes of the study.

Table 34. Logged IFNγ in plasma (pg/ml): time-point comparisons between groups

	Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Week	Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0	-0.01(-0.36 to 0.33)	0.935	0.32(0.01 to 0.63)	0.041*	-0.33(-0.64 to -0.03)	0.033*
12	-0.13(-0.50 to 0.23)	0.473	0.12(-0.22 to 0.45)	0.490	-0.25(-0.59 to 0.09)	0.149

Abbreviations: Logged IFNγ: Logged –transformed value to base 10 of interferon gamma, pg/ml – picograms per millilitre, 95% CI – 95% confidence interval

5.4.5.6 Eotaxin

5.4.5.6.1 Time-point comparisons within groups

Eotaxin values were not normally distributed on Kolmogorov-Smirnov and Shapiro Wilk tests for normality, however log-transformed values were normal. Hence, paired T tests with log-transformed values were used to compare Eotaxin at Week 0 with Week 12 for each group. There were no significant differences in Eotaxin in any group (see Tables 35).

Table 35. Logged Eotaxin in plasma (pg/ml): Time-point comparisons within groups

	Week	Mean (±SE)	Mean Difference (±SE)	P value (2-tailed)
No acupuncture (n = 35)	0	1.78±0.04	0.02±0.03	0.456
	12	1.76±0.04		
Sham acupuncture (n = 34)	0	1.72±0.05	-0.01±0.03	0.777
	12	1.73±0.05		
Real acupuncture (n = 34)	0	1.67±0.05	0.01±0.03	0.703
	12	1.66±0.05		

Abbreviations: Logged Eotaxin - Log-transformed value to base 10 of eotaxin ,pg/ml – picograms per millilitre, n – number of subjects, SE – standard error, SD – standard deviation

5.4.5.6.2 Time-point comparisons between groups

Between-group comparisons for each week were performed with the Independent-Samples T test. There were no significant differences between groups at Week 0 or Week 12 (see Table 36).

Table 36. Logged eotaxin in plasma (pg/ml): time-point comparisons between groups

	Real acupuncture vs no acupuncture		Real acupuncture vs sham acupuncture		Sham acupuncture vs no acupuncture	
Week	Difference (95% CI)	P value	Difference (95% CI)	P value	Difference (95% CI)	P value
0	-0.11(-0.24 to 0.03)	0.112	-0.06(-0.20 to 0.09)	0.435	-0.05(-0.18 to 0.08)	0.417
12	-0.10(-0.23 to 0.04)	0.157	-0.07(-0.21 to 0.07)	0.327	-0.03(-0.16 to 0.10)	0.696

Abbreviations: Logged Eotaxin - Log-transformed value to base 10 of eotaxin , pg/ml – picograms per millilitre, 95% CI – 95% confidence interval* $p < 0.05$ compared to Week 0

5.5 Discussion

A significant reduction was found in total IgE and allergen-specific IgE for house dust mite after acupuncture, but there was no change in allergen-specific IgEs for three regionally relevant grass pollens.

Previous studies on changes in serum IgE after acupuncture in allergic rhinitis were mixed. No significant difference in post-acupuncture IgE was reported by Ng et al, but Rao and Han found a significant decrease in IgE (Ng et al., 2004; Rao and Han, 2006). Magnusson et al tested total IgE and allergen-specific IgE for 5 allergens and found no significant change after acupuncture except for specific IgE for mugwort which significantly decreased (Magnusson et al., 2004). In a small cohort study (n = 22), Lau et al reported a decrease in serum IgE for 64% of subjects post-acupuncture and in 76% of subjects at 2 months follow-up, but to complete the picture, 31% of subjects showed an increase in IgE post-acupuncture, which persisted in 18% of subjects at 2 months follow-up (Lau et al., 1975b). It is noteworthy that the only study to report an unequivocal reduction in IgE provided daily acupuncture treatments 6 days a week for four weeks (Rao and Han, 2006) while the studies which produced mixed results or showed no change in IgE used much less intense treatment regimens (Lau et al., 1975b; Magnusson et al., 2004; Ng et al., 2004). The influence of frequency and duration of acupuncture treatments on outcomes is discussed in Chapter 1.

The decrease in the Rao and Han study in serum total IgE was 21.2% ($p<0.01$*), while in this study the serum total IgE reduction was 17.9% ($p = 0.016$*). In this study a

significant reduction (by 24%) was also found in allergen-specific IgE for house dust mite after acupuncture ($p = 0.035$*).

No significant changes were seen in the neurotrophins NGF and BDNF. This is the first study to examine NGF and BDNF after acupuncture for allergic rhinitis.

Pro-inflammatory neuropeptide SP was significantly reduced after acupuncture in Week 1 and Week 3 but this change was no longer statistically significant at four-week follow-up (Week 12). VIP results showed between-group difference following Week 1 treatment but no significant time-point differences within groups, while CGRP did not change. One previous study also reported a short-term down-regulation in both SP and VIP after acupuncture treatment of allergic rhinitis (Li et al., 2007b). Down-regulation of CGRP after acupuncture has been reported in migraine sufferers, but no previous studies have investigated CGRP in allergic rhinitis (Li, 2001).

There were no significant changes in serum cytokines or salivary ECP (an inflammatory marker) between Week 0 and Week 12.

The planned testing of CGRP, NGF and BDNF in saliva at the same time-points as SP and VIP was abandoned due to levels being undetectable in saliva. Plasma was used instead, but plasma was only collected at Week 0 and Week 12. Clearly there were no changes in CGRP, NGF and BDNF at four-week follow-up, however if there were any short-term changes (similar to those seen in SP and VIP) these changes would not have been captured in this study. Only one human study to date has measured post-acupuncture changes in NGF and BDNF, but these changes were only measured up to 48 hours after treatment (Moldenhauer et al., 2010). In this study NGF levels decreased after one hour but had returned almost to baseline at 24 hours while BDNF decreased slowly, the difference becoming significant at 48 hours (Moldenhauer et al., 2010).

Similarly any potential short-term changes in cytokines after acupuncture (which have been reported in other studies) would not have been observed in this study. A shift in Th1/Th2 balance away from Th2 dominance, which characterises acute allergic response, would suggest a reduction in allergic inflammation. Short-term changes in cytokines (after acupuncture for allergic rhinitis) reported in other studies include down-regulation of Th2 cytokines IL- 1β, IL- 4, IL- 10 and GM-CSF, (Petti et al., 2002; Rao

and Han, 2006; Shiue et al., 2008; Zheng, 2010) There are contradictory findings on the Th1 cytokine IFNγ, with one study reporting up-regulation and another study reporting no change (Rao and Han, 2006; Zheng, 2010). In one study IL- 2, a Th1 cytokine, was down-regulated after acupuncture (Petti et al., 2002). In summary there is some early evidence (although not entirely consistent) to suggest that acupuncture might shift Th1/Th2 balance in allergic rhinitis. In this study no significant changes were seen in either Th2 or Th1 cytokines. Given that there was clear evidence of improvements in symptoms and quality of life in this study, and also in the light of evidence of Th1/Th2 shifts reported in other studies, it is possible that changes in cytokines may have occurred in this study but were not captured, due to the time-points measured. All previous studies reporting cytokine changes in allergic rhinitis after acupuncture measured immediately or very soon after treatment. It is possible that no changes in cytokines were seen at four-week follow-up because they would have been short-lived effects which would have washed out long before the measurements were taken.

Notwithstanding the lack of changes in serum Th1 and Th2 cytokine, the decrease in total IgE and house dust mite specific IgE does suggest a shift in Th1/Th2 balance (Deo et al., 2010). This down-regulation in IgE was measured at four-weeks follow-up, so the IgE changes suggest some form of immunomodulation beyond any short-term changes in neurotrophins, neuropeptides or cytokines.

5.6 Conclusions

In this study total IgE and allergen-specific IgE for house dust mite were significantly decreased at four-week follow-up, but allergen-specific IgE for three regionally relevant grass pollens were unchanged. Persistent allergic rhinitis is more associated with allergy to house dust mite, and pollen sensitivity tends to characterise intermittent allergic rhinitis. This study included adults with persistent allergic rhinitis, but excluded those with intermittent rhinitis. A statistically significant decrease in allergen-specific IgE for house dust mite in adults with persistent allergic rhinitis is therefore likely to be clinically significant.

Down-regulation of SP was seen only after real acupuncture, but this effect was short-lived and by four-week follow-up SP levels had returned to more than half of baseline levels. VIP levels were significantly lower after real acupuncture than after sham acupuncture 18 to 24 hours post-treatment in Week 1, however there were no significant differences between time-points within any group. No changes were seen in CGRP, NGF, BDNF, ECP or cytokines.

In future studies of acupuncture for allergic rhinitis, it is recommended that sample collections for biomarkers focus more attention on pre-treatment versus post-treatment (e.g.: pre-treatment, 6 to 8 hrs post-treatment, 18 to 24 hours post-treatment, 48 hours post-treatment, 72 hours post-treatment) in the first three weeks of treatment. In this study significant improvements in daily symptoms were seen in the third week of treatment, so it may not be necessary to treat twice weekly for 8 weeks in future studies which are only investigating short-term changes in neurotrophins, neuropeptides or cytokines.

Chapter 6. Baseline clinical and immunological measurements: healthy controls vs adults with allergic rhinitis

6.1 Abstract

Background: A small cohort (n = 20) of non-atopic healthy volunteers was recruited to provide comparisons between non-atopic adults and atopic adults' baseline measurements.

Objectives: To compare clinical examination and biomarker results from a small group of non-atopic healthy adults with similar baseline measurements from atopic adults.

Methods: During a single visit to the allergy clinic, blood, saliva and nasal fluid samples were collected from the non-atopic healthy control group. At the same time clinical examinations were conducted, namely peak nasal inspiratory flow measurement and rhinoscopic examination to assess swelling of the inferior turbinates. Data from the healthy control group was then compared with data from the three atopic groups.

Results: Peak nasal inspiratory flow was 28% lower in atopic adults compared with healthy volunteers, while inferior turbinate obstruction was 45% greater. Total IgE was 4 times higher in atopic adults while allergen-specific IgE for house dust mite was 73 times higher than in healthy controls. BDNF was 23 times higher in atopic adults, but surprisingly, NGF was significantly lower in atopic adults than in healthy controls. There were no significant differences in pro-inflammatory neuropeptides, ECP or cytokines except for eotaxin.

Conclusions: The differences between atopic individuals and non-atopic adults in objective nasal patency measures, total and allergen-specific IgE and BDNF were all consistent with current understanding of the nature of allergy based on published literature. However the lower levels of NGF and eotaxin in the atopic group, and the lack of difference in inflammatory markers such as ECP and the pro-inflammatory neuropeptides were unexpected and not consistent with previous studies.

6.2 Introduction

In order to compare immunological measurements between atopic adults with allergic rhinitis and healthy adults, a cohort of 20 healthy volunteers (also aged 18 to 45 years) was recruited between August and November, 2012. Any volunteers who showed positive skin prick tests for house dust mite, Bermuda grass, Johnson grass and/or Bahia grass were excluded from the healthy control group. No measures of symptoms (such as iTNSS) or quality of life associated with allergic rhinitis (such as MiniRQLQ) were undertaken as this group was asymptomatic.

6.3 Methods

From each healthy volunteer samples of blood, saliva and nasal fluids were collected at the allergy clinic during a single visit. The mean level of obstruction caused by swelling of the inferior turbinate (as a percentage) was estimated during rhinoscopic inspection by the allergologist and peak nasal inspiratory flow (PNIF) was measured by the clinical assistant. Details of collection, handling and analysis of samples are given in Chapter 2.

6.4 Results

6.4.1 Clinical examinations

6.4.1.1 Peak nasal inspiratory flow: comparisons between atopic adults and healthy controls

PNIF was significantly lower (28% lower) in atopic adults than in healthy controls ($p = 0.018$*) (see Table 1).

6.4.1.2 Mean inferior turbinate obstruction: comparisons between atopic adults and healthy controls

Mean inferior turbinate obstruction was significantly higher (45% higher) in atopic adults than in healthy controls ($p = 0.001$**) (see Table 1).

6.4.1.3 Negative correlation between mean inferior turbinate obstruction and peak nasal inspiratory flow (PNIF)

Spearman's rho test revealed a significant negative correlation (correlation coefficient = - 0.220; p = 0.004*) between mean lower turbinate obstruction and peak nasal inspiratory flow (when data from 150 atopic and 19 non-atopic individuals was combined).

6.4.2 Laboratory analysis

6.4.2.1 Total and allergen-specific immunoglobulin E (IgE)

6.4.2.1.1 Total IgE: comparisons between atopic adults and healthy controls

Serum levels of total IgE were significantly higher (4 times higher) in atopic adults than in healthy controls (p = 0.027*) (see Table 1).

6.4.2.1.2 Allergen-specific IgE for three regionally relevant grass pollens: comparisons between atopic adults and healthy controls

There was no significant difference between atopic adults and healthy controls in serum levels of allergen-specific IgE for Bahia grass (p = 0.065), for Johnson's grass (p = 0.068) or for Bermuda grass (p = 0.094) (see Table 1).

6.4.2.1.3 Allergen-specific IgE for house dust mite: comparisons between atopic adults and healthy controls

Serum levels of allergen-specific IgE for house dust mite were significantly higher (73 times higher) in atopic adults than in healthy controls (p = 0.001**) (see Table 1)

Table 1. Comparison of clinical characteristics of healthy controls and atopic adults

	Healthy controls		Atopic adults			
	n	Mean ± SE	n	Mean ± SE	Mean difference± SE	P value (2-tailed)
Mean inferior turbinate obstruction (%)	20	47± 5.0	149	68± 1.4	21±4.3	0.001**
Peak nasal inspiratory flow rate (PNIF) (L/min)	20	112± 11.0	149	81± 3.5	-31±10.7	0.018*
Total IgE (kU/L)	20	57± 16.4	106	285± 43.8	227± 101.4	0.027*
Allergen-specific IgE Bahia grass (kU/L)	20	0.35± 0.0	106	8± 1.8	8± 4.1	0.065
Allergen-specific IgE Johnsons grass (kU/L)	20	0.35± 0.0	106	4± 0.9	4± 2.0	0.068
Allergen-specific IgE Bermuda grass (kU/L)	20	0.35± 0.0	106	6± 1.3	5± 3.1	0.094
Allergen-specific IgE dustmite (kU/L)	20	0.37± 0.0	106	27± 3.1	27± 7.1	0.001**
NGF (plasma) (pg/ml)	20	10.8± 0.8	105	3.8± 0.38	- 6.9± 1.0	0.001**
BDNF (plasma) (pg/ml)	20	92± 11.4	102	2154± 196.9	2062± 446.1	0.001**
SP (saliva) (pg/ml)	20	386± 91.8	109	417± 143.5	31± 338.4	0.927
VIP (saliva) (pg/ml)	20	43± 10.3	108	48± 12.2	5± 28.7	0.851
CGRP (plasma) (pg/ml)	20	41± 2.8	106	29± 2.8	-12± 6.5	0.076
IL-2 (plasma) (pg/ml)	20	23± 7.3	100	8± 3.7	- 15± 8.9	0.096
IL-4 (plasma) (pg/ml)	20	27± 14.6	98	23± 5.8	- 3± 14.4	0.812
IL-10 (plasma) (pg/ml)	20	12± 4.5	100	18± 3.8	6± 8.8	0.523
IL-12(p70) (plasma) (pg/ml)	20	29± 12.8	110	104± 43.2	75± 101.7	0.461
IFNγ (plasma) (pg/ml)	20	77± 42.2	107	34± 8.4	- 43± 26.4	0.106
Eotaxin (plasma) (pg/ml)	20	202± 25.2	106	64± 44.3	- 138± 14.6	0.001**
ECP (saliva) (ng/ml)	19	6.5± 1.5	101	3.5± 6.3	- 3.0± 1.6	0.059

Abbreviations: SE – standard error, n – number of subjects, L/min – litres per minute, kU/L - kilounits per litre, pg/ml – picograms per millilitre, ng/ml – nanograms per millilitre, IgE – immunoglobulin E, NGF – nerve growth factor, BDNF – brain-derived neurotrophic factor, SP – substance P, VIP – vaso-active intestinal peptide, CGRP – calcitonin gene-related peptide, IL – interleukin, IFNγ- interferon gamma, ECP – eosinophilic cationic protein

* $p < 0.05$, ** $p < 0.001$

6.4.2.2 Neurotrophins

6.4.2.2.1 NGF: comparisons between atopic adults and healthy controls

Plasma levels of NGF were significantly lower in atopic adults than in healthy controls (p = 0.001**) (see Table 1).

6.4.2.2.2 BDNF: comparisons between atopic adults and healthy controls

Plasma levels of BDNF were significantly higher (23 times higher) in atopic adults than in healthy controls at baseline (p = 0.001**) (see Table 1).

6.4.2.3 Pro-inflammatory neuropeptides

SP, VIP and CGRP: comparisons between atopic adults and healthy controls

Between atopic adults and healthy controls there were no significant differences between salivary SP levels (p = 0.927), salivary VIP levels (p = 0.851) or plasma CGRP levels (p = 0.076) (see Table 1).

6.4.2.4 Other inflammatory markers

ECP: comparisons between atopic adults and healthy controls

There was no significant difference between salivary ECP levels in atopic adults and healthy controls (p = 0.059) (see table 1).

6.4.2.5. Cytokines

6.4.2.5.1 IL-2, IL-4, IL-10, IL-12(p70), IFNγ: comparisons between atopic adults and healthy controls

Between atopic adults and healthy controls there were no significant differences between plasma levels of IL-2 (p = 0.096), IL- 4 (p = 0.812), IL- 10 (p = 0.523), IL-12(p70) (p = 0.461) or IFNγ (p = 0.106) (see Table 1).

6.4.2.5.2 Eotaxin: comparisons between atopic adults and healthy controls

Plasma levels of Eotaxin were significantly lower in atopic adults than in healthy controls (p = 0.001**).

6.5 Discussion

6.5.1 Clinical examinations

Since nasal obstruction is a major symptom of persistent allergic rhinitis, it would reasonable to expect that healthy individuals would have less nasal obstruction and more nasal patency than adults with allergic rhinitis. Atopic adults showed 45% more obstruction at the level of the inferior turbinates (p = 0.001**) and 28% less peak nasal inspiratory flow (p = 0.018*) than healthy controls (see Table 1). There was a significant negative correlation (correlation coefficient = - 0.220; p = 0.004*) between mean lower turbinate obstruction and peak nasal inspiratory flow rate.

6.5.2 IgE

Since total and allergen-specific IgE would generally be expected to be higher in atopic adults than in non-atopic individuals, it was not surprising to find that total IgE was 4 times higher and allergen-specific IgE for house dust mite was 73 times higher in atopic adults, than in healthy controls. However there were no significant differences in allergen-specific IgE for three local grass pollens between atopic and non-atopic adults. Given that non-atopic subjects were required to have negative skin prick tests for the three grass pollens and house dust mite to be included in the healthy group, it might be expected that no subjects in the non-atopic group would show any detectable levels of specific IgE for pollens or dust mite. In fact one of the 20 non-atopic adults did show

negligible levels of allergen-specific IgE to Bahia grass (0.38 kU/L – detectable threshold 0.35 kU/L) and to Bermuda grass (0.42 kU/L – detectable threshold 0.35 kU/L) while another individual in the non-atopic groups showed detectable allergen-specific IgE for house dust mite (0.69 kU/L – detectable threshold 0.35 kU/L). In contrast, in the atopic group 43% had detectable levels of allergen-specific IgE for Bahia grass, 41% for Johnson's grass and 42% for Bermuda grass. The lack of statistical significance may be due to the small numbers in the non-atopic group and the high variability of data in the atopic group.

6.5.3 Neurotrophins

Plasma levels of NGF were significantly lower in atopic adults than in healthy controls (p = 0.001**) (see Table 1). This result was surprising given that elevated levels of NGF have been consistently reported to be associated with allergic rhinitis (Bresciani et al., 2009; Pfaar et al., 2009; Raap and Braunstahl, 2010; Raap et al., 2008). High variability between individuals (both atopic and non-atopic) has previously been reported (Bonini et al., 2013; Bonini et al., 1996). For example in a sample of 9 patients with allergic rhinitis/rhinoconjunctivitis, serum NGF levels ranged from 0 to 1,213.4 pg/ml (Bonini et al., 1996). In another study, serum NGF levels were compared between Olympic athletes (mean 368.3±776.3 pg/ml) and healthy sedentary non-atopic controls (mean 174.1±483.7 pg/ml) (Bonini et al., 2013). In the same study, however, there was no significant difference in serum NGF between atopic and non-atopic athletes (Bonini et al., 2013). Physical exercise and stress have been shown to produce elevated serum levels of NGF, quite independent of any allergic response (Bonini et al., 2013). It is possible that these, or other unknown factors, contributed to the higher levels of plasma NGF in healthy adults than in atopic individuals in this study. There may also be differences in measurements between this study and previous studies as a result of measuring plasma levels rather than serum levels of NGF.

Plasma levels of BDNF were significantly higher (23 times higher) in atopic adults than in healthy controls at baseline (p = 0.001**). BDNF levels have been reported to rise sharply in response to nasal allergen challenge in atopic individuals but not in healthy controls (Pfaar et al., 2009)

6.5.4 Pro-inflammatory neuropeptides

There were no significant differences between atopic adults and healthy controls in salivary levels of SP and VIP and in plasma levels of CGRP. In this study and one previous study, decreases in SP and VIP after acupuncture treatment have been associated with clinical improvement, hence lower levels of SP and VIP might have been expected in healthy non-atopic individuals (Li et al., 2007b). The measurement of pro-inflammatory neuropeptides in healthy subjects is discussed further in Chapter 7.

6.5.5 Other inflammatory markers: ECP

There was no significant difference between atopic adults and healthy controls in salivary levels of ECP. Again, since ECP is associated with high levels of eosinophilic infiltration of the nasal mucosa such as that seen in persistent allergic rhinitis, ECP would have been expected to be higher in atopic than in non-atopic healthy individuals.

6.5.6 Cytokines

There were no significant differences between atopic adults and healthy controls in plasma levels of IL- 2, IL- 4, IL- 10, IL- 12(p70) and IFNγ. However the plasma levels of eotaxin were significantly lower in atopic adults than in healthy controls ($p = 0.001^{**}$). This was unexpected as eotaxin is a marker of inflammation, hence eotaxin would generally have been expected to be higher in the atopic group.

6.6 Conclusions

Given the prominence of nasal obstruction as a symptom of allergic rhinitis, to find reduced nasal patency and nasal air flow in atopic subjects compared to non-atopic subjects is not unexpected. It also makes intuitive sense that greater turbinate swelling should be linked to less nasal air flow, and this was confirmed.

Higher levels of total IgE were seen in atopic subjects which is consistent with previous studies (Deo et al., 2010). Specific IgE to house dust mite was 73 times higher in adults with persistent allergic rhinitis than in healthy non-atopic individuals. Also 88% of the atopic subjects recruited showed detectable levels of specific IgE to house dust mite, in contrast to only 41-43% having detectable levels of specific IgE for the three local grass pollens. This difference was due to the study's inclusion criteria which required a two-year history of persistent allergic rhinitis, and excluded those with only intermittent

(seasonal) rhinitis which would be more likely to be sensitive to grass pollens. Only four people who satisfied the inclusion criteria, and were accepted for the study, were found to have specific IgE for all three grass pollens but not to house dust mite.

Although only one of the 20 subjects in the non-atopic group (5%) showed detectable levels of specific IgE to grass pollens compared to 41-43% in the atopic group, there was no significant difference between the groups. This lack of difference may be due to high variability in the atopic group and low numbers in the healthy group.

NGF levels have been reported to exhibit high variability between individuals, whether atopic or non-atopic. Both stress and physical exercise have been shown to elevate NGF levels in both atopic and non-atopic individuals, independent of allergic inflammatory response. It is unclear whether stress or physical exercise or other unknown factors were responsible for the non-atopic group showing significantly higher NGF levels than the atopic group, when the opposite would have been anticipated.

BDNF levels, on the other hand, were 23 times higher in atopic adults than in the non-atopic group, which is consistent with previous studies.

In allergic inflammation, on the basis of previous studies, it would be anticipated that pro-inflammatory neuropeptides and other inflammatory markers would be higher in atopic individuals than in non-atopic healthy controls. However there were no significant differences between atopic and non-atopic adults in the pro-inflammatory neuropeptides SP, VIP and CGRP, or in the inflammatory marker ECP. Another inflammatory marker, eotaxin was unexpectedly higher, not lower, in the non-atopic group than the atopic group. There were also no significant differences in plasma levels of pro-inflammatory cytokines and Th1 and Th2 cytokines between the two groups. The reasons for these unexpected findings are unknown.

Chapter 7. Baseline immunological measurements in healthy adults: nasal secretions vs saliva and/or plasma

7.1 Abstract

Background: Saliva samples have been used throughout this study instead of nasal lavage fluids as the collection procedure is less invasive for subjects. Saliva sampling was chosen based on previous studies suggesting that saliva provides an accurate reflection of mucosal immune activity (Gleeson et al., 1995). To test the adequacy of saliva sampling for this purpose, un-stimulated natural nasal fluids were collected from 20 non-atopic healthy volunteers, as well as saliva and peripheral venous blood. Levels of the pro-inflammatory SP, VIP and CGRP as well as a marker of eosinophil survival, ECP were then compared between nasal fluid and saliva and/or plasma for these four biomarkers.

Methods: Nasal fluids, saliva and plasma were assayed with enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions. Sample collection and handling is detailed in Chapter 2.

Results: There was high variability between subjects in this study in nasal fluid levels of SP, VIP, CGRP, ECP and NGF.

Discussion: There was no correlation between nasal fluid levels and salivary levels of SP, VIP or ECP. However there was a positive correlation between plasma levels of CGRP and nasal fluid levels. High variability has also been reported in previous studies measuring SP, VIP and CGRP.

Conclusions: No firm conclusions can be drawn from these data on correlations due to small sample size, high numbers of out of range values and small sample volumes (limiting the number of samples available for testing), and high variability between subjects.

7.2 Introduction

The collection of un-stimulated natural nasal fluids using nasal tampons was a novel method for non-invasive sampling of nasal fluids introduced in this study. The nasal tampons only became available after the completion of the treatment phase of the study, hence nasal fluids were only collected from 20 non-atopic healthy volunteers after the rest of the study was completed. The objective of collecting nasal fluids was to compare levels of SP, VIP and ECP in nasal fluids with salivary levels, and to compare plasma and nasal fluid levels of CGRP. (Plasma was used for CGRP due to assays of saliva showing CGRP below detectable levels.)

Due to small volumes of nasal fluids being collected from most subjects, it was not possible to assay all analytes in all subjects. Samples tested and the results which were available for analysis (after out of range values were removed) are detailed in Table 1.

Table 1. Nasal fluid collection, testing and analysis in 20 healthy adults

	Samples collected	Samples tested	Out of range (low)	Out of range (high)	Results analysed
SP (nasal fluids) (pg/ml)	20	19	0	0	19
VIP (nasal fluids) (pg/ml)	20	6	0	0	6
CGRP (nasal fluids) (pg/ml)	20	18	6	0	12
ECP (nasal fluids) (ng/ml)	20	20	3	0	17
NGF (nasal fluids) (pg/ml)	20	4	4	0	0

Abbreviations: SP – Substance P, VIP – vaso-active intestinal peptide, CGRP – calcitonin gene-related peptide, ECP – eosinophilic cationic protein, pg/ml – picograms per millilitre, ng/ml - nanograms per millilitre

7.3 Methods

Methods including sample collection and handling, and laboratory analysis are detailed in Chapter 2.

7.4 Results

There was considerable variability between healthy individuals in nasal fluid levels of SP, VIP, CGRP and ECP (see Table 2 and Table 3). No previous studies which have measured these substances in natural nasal fluids from healthy adults are available for comparison.

Table 2. Nasal fluid levels of SP, VIP, CGRP and ECP in healthy adults

	n	Mean (±SE)	Range
SP (nasal fluids) (pg/ml)	19	86.6±14.8	21.8 - 273.8
VIP (nasal fluids) (pg/ml)	6	3.2±0.6	1.1 - 4.9
CGRP (nasal fluids) (pg/ml)	12	3.0±0.2	2.0 - 4.8
ECP (nasal fluids) (ng/ml)	17	17.4±4.6	0.1 - 75.5

Abbreviations: SP – Substance P, VIP – vaso-active intestinal peptide, CGRP – calcitonin gene-related peptide, ECP – eosinophilic cationic protein, pg/ml – picograms per millilitre, ng/ml - nanograms per millilitre, n – number of subjects , SE – standard error

Table 3. Comparison of nasal fluid levels and saliva or plasma levels of SP, VIP, CGRP and ECP in 20 healthy adults

Subject ID	SP (saliva) (pg/ml)	SP (nasal fluid) (pg/ml)	VIP (saliva) (pg/ml)	VIP (nasal fluid) (pg/ml)	CGRP (plasma) (pg/ml)	CGRP (nasal fluid) (pg/ml)	ECP (saliva) (ng/ml)	ECP (nasal fluid) (ng/ml)
LA 01	232.6	142.1	5.3	4.9	34.7	2.7	0.8	43.3
CC 02	139.5	21.8	8.6		37.3	OORL	16.4	2.9
JF 03	294.7	37.8	15.0		24.5	OORL	0.4	OORL
JW 04	91.9	29.9	38.4		51.0	OORL	8.1	OORL
KB 05	112.1	52.7	OORL		42.6	OORL	OORL	75.5
DL 06	228.2	99.6	34.7		31.1	OORL	14.0	22.9
RT 07	799.2	25.6	18.4		37.4		2.9	OORL
CK 08	243.8	51.5	OORL	4.4	52.4	3.0	OORL	9.6
RM 09	688.0	75.6	50.4	3.3	27.2	2.6	1.6	25.4
GB 10	410.8	106.2	62.3		40.4	2.2		45.1
AH 11	1805.3	29.8	162.3	1.1	48.4	3.7	23.6	3.5
NF 12	487.0	46.5	19.2	2.0	31.0	2.3	1.5	10.8
MD 13	145.2	74.5	97.2		72.4	3.7	14.1	14.4
RN 14	304.2	96.0	15.9		39.2	2.0	1.2	0.2
AB 15	323.2		11.5	3.2	35.2	2.4	5.2	2.3
WH 16	OORL	78.5	118.0		41.1	2.8	8.6	43.6
AK 17	OORL	61.7	83.1		68.2	4.8	7.5	6.9
SM 18	240.9	166.3	9.0		31.4	OORL	8.4	19.6
RD 19	309.5	174.9	4.4		35.3	3.4	4.0	2.1
NH 20	857.7	273.8	94.3		37.9		5.5	19.5
	Mean ±SE	**Mean ±SE**	**Mean ±SE**	**Mean ±SE**	**Mean ±SE**	**Mean ±SE**	**Mean ±SE**	**Mean ±SE**
	385.9± 91.8	86.6± 14.8	44.7± 10.6	3.2±0.6	40.9±2.8	3.0±0.2	6.5±1.5	17.4±4.6

Abbreviations: SP – substance P, VIP – vaso-active intestinal peptide, CGRP – calcitonin gene-related peptide, ECP – eosinophilic cationic protein, n – number of subjects, pg/ml – picograms per millilitre, ng/ml – nanograms per millilitre, OORL – out of range (low)

7.5 Discussion

7.5.1 Correlations between nasal fluid levels and saliva or plasma levels of SP, VIP, CGRP and ECP in healthy adults

There was no correlation between nasal fluid levels and saliva levels of SP (correlation coefficient 0.076; p = 0.756) or VIP (correlation coefficient -0.771; p = 0.072), or ECP (correlation coefficient 0.003; p = 0.991). However there was a positive correlation between plasma levels of CGRP and nasal fluid levels (correlation coefficient 0.629; p = 0.028*).

7.5.2 Comparisons with levels of SP, VIP and CGRP measured in saliva, nasal secretions, plasma and serum in previous studies

Comparisons between levels of SP, VIP and CGRP in various body fluids in previous studies are complicated by the differences in units of measurement used (eg: picograms per millilitre, picomoles per litre, picomoles per milligram of total protein). To facilitate comparison, where original figures were given in nanograms per millilitre these have been converted into picograms per millilitre. Across studies there is a fairly broad range of values (see Table 4). For example SP salivary values range from 96 to 385.9 pg/ml, plasma SP from 42.87 to 222 pg/ml and plasma CGRP from 33.74 to 1150 pg/ml. Some of this variability may be due to different definitions of “healthy subjects”. For example when migraine sufferers were being compared with healthy subjects, “healthy” meant anyone who was free of migraines (Gupta et al., 2009). In this study “healthy” meant tested negative on allergy tests such as skin prick tests and RAST tests for three local grass pollens and house dust mite. Another possibility is that SP, VIP and CGRP levels may vary significantly over time in healthy subjects, and so one-off measurement is subject to high variability. This could be tested by sampling repeated measures in the same group of healthy subjects over a period of time. Also, due to circadian variability, sampling should be taken at the same time of day (Trasforini et al., 1991).

Table 4. Levels of SP, VIP, CGRP in healthy controls in studies

Author/ year	Measured in		SP		VIP		CGRP
McDonald 2014	saliva	n=20	385.9±91.8 pg/ml	n=20	42.50±10.26 pg/ml		
Boras 2010	saliva	n=22	95±26 pg/ml			n=22	150±28 pg/ml
Dawidson 1998	saliva	n=8	5.6±2.2 pmol/L	n=8	12.0±7.8 pmol/L	n=8	6.2±5.3 pmol/L
Jang 2011	saliva	n=36	177.0±106.2 pg/ml			n=36	301.5±2188.9 pg/ml
Takeyama 1990	saliva	n=?	7 pmol/L				
Bellamy 2006	saliva			n=15	40 pmol/mg total protein	n=15	10 pmol/mg total protein
Cady 2009	saliva					n=22	56±4 pmol/mg total protein
McDonald 2014	nasal secretions	n=19	86.6±14.8 pg/ml	n=6	3.15±0.58 pg/ml	n=12	2.95±0.23 pg/ml
Chaen 1993	nasal secretions	n=?	224 pmol/L	n=?	41.6 pmol/L		
Chaen 1993	plasma	n=?	3.04 pmol/L	n=?	1.04 pmol/L		
Takeyama 1990	plasma	n=?	~2.4 pmol/L				
McDonald 2014	plasma					n=20	40.9±2.8 pg/ml
Jang 2011	plasma	n=36	105.0±67.1 pg/ml			n=36	136.2±92.5 pg/ml
Cernuda-Morollon 2013	plasma					n=31	33.74 pg/ml
Maintz 2011	plasma	n=42	170±10 pg/ml	n=42	2870±1360 pg/ml	n=40	920±60 pg/ml
Gupta 2009	plasma					n=50	940-1150 pg/ml
Dalby 1997	plasma	n=20	222±45 pg/ml				
Trasforini 1991	plasma					n=8	11.7±0.4 – 18.1±1.5 pmol/L
Li 2007	plasma		42.87±12.13 pg/L≠		36.4±11.25 pg/L≠		
Yoshida 1992	serum					n=39	< 100.8 pmol/L
Boras 2010	serum	n=22	151±15 pg/ml			n=22	260±43 pg/ml

Abbreviations: SP – substance P, VIP – vaso-active intestinal peptide, CGRP – calcitonin gene-related peptide, n – number of subjects, pg/ml – picograms per millilitre, pmol/L – picomoles per litre, pmol/mg – picomoles per milligram

≠ pg/L – picograms per litre is probably a typographical error for pg/ml – picograms per millilitre

References: (Bellamy et al., 2006; Boras et al., 2010; Cady et al., 2009; Cernuda-Morollon et al., 2013; Dalby et al., 1997; Dawidson et al., 1998; Gupta et al., 2009; Jang et al., 2011; Li et al., 2007b; Maintz et al., 2011; Takeyama et al., 1990; Trasforini et al., 1991; Yoshida et al., 1992)

Results from this study are shaded above

7.6 Conclusions

It is difficult to draw any firm conclusions from these data on correlations due to the small sample size, the small volumes of nasal fluids collected, and the relatively high number of out of range (low) values. To clarify what constitutes typical values of SP, VIP, CGRP, ECP, NGF and BDNF in various forms of nasal fluids, saliva, plasma and serum in healthy subjects, further studies are needed using a standardised set of inclusion and exclusion criteria for “healthy subjects”, an adequate sample size, standardised collection times (to limit circadian variations) and repeated measures over time.

Chapter 8: Discussion, conclusions and recommendations for future research

8.1 Introduction

8.1.1 The burden of disease: prevalence and cost

Estimates of the worldwide prevalence of allergic rhinitis range between 10% and 20% with higher prevalence in some age groups (AccessEconomics, 2007; Bousquet et al., 2008; Wallace et al., 2008). In Australia, the prevalence of allergic rhinitis is estimated to be 16.8% of the population, or 3.7 million people (ABS, 2013). This figure has risen from 13.9% in 1995, and prevalence continues to increase both in Australia and worldwide (ABS, 1996; AccessEconomics, 2007; Dykewicz and Hamilos, 2010). The highest prevalence by age group in Australians is found in 15 to 54 year-olds, ranging from 18.8% to 22.5% (ABS, 2013). For Australian 15-24 years old, "hayfever and allergic rhinitis" was the most frequently reported long-term condition in the Australian Health Survey (2001-2012) (ABS, 2013). The cost of allergies (excluding allergic asthma) to the Australian health system in 2007 was estimated to be $349.3 million, while productivity losses for all allergies combined were estimated at $6.6 billion (AccessEconomics, 2007). No separate figures for allergic rhinitis are available. Allergic rhinitis is a significant burden to the Australian community in quality of life and wellbeing, impaired performance, loss of productivity and health care costs.

8.1.2 Summary of previous studies on acupuncture treatment for allergic rhinitis

To date, four systematic reviews have been published on the clinical efficacy of acupuncture for allergic rhinitis (Lee et al., 2009; Passalacqua et al., 2006; Roberts et al., 2008; Xiao et al., 2009). The earliest systematic review concluded that there was insufficient evidence to support the use of acupuncture for allergic rhinitis, while the most recent review concluded that acupuncture is a safe and effective treatment for allergic rhinitis, but more high quality RCTs are needed. In 2009, evidence of acupuncture efficacy for persistent (perennial) allergic rhinitis was regarded as stronger than for intermittent (seasonal) allergic rhinitis (Lee et al., 2009).

Currently 26 relevant RCTs on acupuncture treatment for allergic rhinitis have been published, 9 of which were in English, 12 in Chinese, 4 in German and 1 in Korean. Since the most recent systematic review was written, another pragmatic study involving

5,237 subjects has been published in English which included two randomised arms and one very large non-randomised arm (Brinkhaus et al., 2008). Two large multi-centre RCTs have also been published since the latest systematic review, one on moderate to severe persistent allergic rhinitis and the other on intermittent (seasonal) allergic rhinitis (Brinkhaus et al., 2013; Choi et al., 2013). These studies have strengthened the evidence for the safety and effectiveness of acupuncture for persistent allergic rhinitis and intermittent allergic rhinitis respectively.

Although the primary goal of this study was to investigate immune modulation in response to acupuncture treatment for persistent (perennial) allergic rhinitis, as it was a randomised, controlled, subject-and-assessor-blinded clinical trial, the results of the study include further evidence to support the efficacy and safety of acupuncture for this condition.

8.2 Population

8.2.1 Potential sources of bias in sample recruitment

8.2.1.1 Gender bias

Of the subjects who were screened for the trial, 73.0% were female while 73.6% of those who completed the trial were female. In contrast, the population of Australians with allergic rhinitis is estimated to be 51.8% female (ABS, 2013). The sample for this study was a "convenience sample" - consisting of those who responded to the staff/student emails sent out at Griffith University, newspaper and television coverage of the study and other advertising. This gender bias simply reflects the population who volunteered for the study. Although no formal statistics are available, anecdotal evidence from Australian acupuncturists suggests that the population of acupuncture patients in Australia is generally around 70% female. So it is possible that this gender bias in our sample represents a greater acceptance of acupuncture treatment by women. This discrepancy between the gender balance in our sample, and the gender balance in the Australian population of allergic rhinitis sufferers limits the degree to which the results of the study can be generalised.

8.2.1.2 Age bias

Since only adults between 18 and 45 years were selected for the study, the results cannot be generalised to all age groups within the Australian community. The latest Australian Health Survey statistics show that only 63.2% of Australians with allergic rhinitis are between the ages of 15 and 45 (ABS, 2013).

8.2.1.3 Randomisation bias

A total of 9 subjects who completed the trial in the no acupuncture group were re-enrolled and randomised to either the real or sham acupuncture groups (but not to the no acupuncture group for a second time). Of these 9 re-enrolled subjects, 7 completed the second course of treatment in the study (6.4% of completed subjects). Hence this randomisation bias would have been unlikely to have a large impact on final results.

8.2.1.4 Disease bias

Since asthma sufferers who also had allergic rhinitis were excluded from the study, this would produce a disease bias in the sample. It has been reported that 40% of allergic rhinitis patients also have allergic asthma, while 80-95% of allergic asthma patients also have allergic rhinitis (Braunstahl, 2009).

8.2.1.5 Sample size

Although the sample size which was calculated in the power calculation for the study was achieved (110 with 108 required), the drop-out rate was 25.6%. Assumptions in the power calculation only allowed for a 10% drop-out rate in each group. Although some statistically significant changes were observed, it is possible that the study was under-powered.

Since the power calculation was based on what sample size would be needed to detect improvement in clinical symptoms (which was achieved), it is possible that the study was under-powered to detect potential changes in biomarkers such as immunoglobulins, neurotrophins, neuropeptides and cytokines.

8.2.2 Baseline comparison between 3 trial groups

There were no statistically significant differences between groups at baseline in gender, age, symptom severity, mean inferior turbinate obstruction, peak nasal inspiratory flow, total IgE, specific IgEs, neurotrophins, pro-inflammatory neuropeptides and ECP. As for the cytokines, there were no significant differences between groups in levels of eotaxin. However there were some significant between-group differences in most of the cytokines, with the no acupuncture group being higher than the real and sham acupuncture groups in levels of IL- 2, IL- 4, IL- 10, IL- 12 (p70) and IFNγ.

Hence, the three randomised groups were well-matched with respect to the parameters measured, except for the levels of cytokines. Since none of these cytokines showed any significant differences within groups between Week 0 and Week 12, it is unlikely that these baseline cytokine differences would have had any significant impact on the results of the study. In addition, the between-group differences at Week 12 largely reflected the differences which were seen already at Week 0, namely unusually high levels of IL- 2, IL- 4, IL- 10, IL- 12 (p70) and IFNγ.

8.2.3 Baseline comparison between atopic and healthy adults

Peak nasal inspiratory flow was 28% lower in atopic adults compared with healthy volunteers, while inferior turbinate obstruction was 45% greater. Total IgE was 4 times higher in atopic adults while allergen-specific IgE for house dust mite was 73 times higher than in healthy controls. BDNF was 23 times higher in atopic adults. These differences were all consistent with previous studies.

Surprisingly, however, NGF was significantly lower in atopic adults than in healthy controls. Some recent studies on NGF have shown that physical exercise and stress can both elevate levels of NGF, however it is unclear whether these factors were responsible for the unexpectedly high levels of NGF in the healthy control group.

Eotaxin was also lower in the atopic group, not higher, as might be expected for an inflammatory marker. There were no significant differences in pro-inflammatory neuropeptides, ECP or cytokines other than eotaxin.

Since eotaxin, ECP and the pro-inflammatory neuropeptides SP, VIP and SP are generally elevated in allergic inflammation, these results were surprising and not consistent with previous studies. The reasons for these unexpected results are not known.

8.3 Intervention

8.3.1 Acupuncture point selection

The acupuncture points selected for the real acupuncture group in this study were based on a review of previous research studies as well as contemporary and historical acupuncture texts. All of the points used for the real acupuncture group were well-supported by the evidence from these three sources (see Chapter 2.4.1 for details). A joint Chinese/Korean multi-centre study published in 2013 used exactly the same acupuncture points for their study of acupuncture for persistent allergic rhinitis as were selected for this study (with the addition of one point) (Choi et al., 2013).

8.3.2 Frequency and duration of treatments

The frequency and duration of treatment sessions was also based on a review of previous studies, which showed that there is a clear difference in outcomes between studies which have treated at least twice weekly for at least six weeks, and those studies which have used less than this. The decision to extend the duration to 8 weeks (with twice weekly treatments) was based on the success of a study by Xue et al (Xue et al., 2007).

8.3.3 Circadian variability

Circadian variations both in the immune system and in responses to acupuncture were an important consideration in the design of the study. All acupuncture treatments were given between 6am and 12 md to limit the degree of circadian variation, and all saliva samples collected at the acupuncture clinic were collected within the same time-frame. Blood collections at the pathology collection centre and other testing conducted at the allergy clinic were not able to be controlled in the same way due to operating hours of

both centres. Generally blood collections and allergy clinic appointments occurred around 4 to 5pm.

8.3.4 Responders and non-responders

It has been suggested that acupuncture is only effective for some people and that this group of "responders" is 70% of the population (Longhurst, 2012). After an examination of clinical symptom and quality of life data from the group who received real acupuncture in this study, it was found that 31 out of 36 subjects (86.1%) were responders while 5 (13.9%) were non-responders. An analysis of individual patient data from previous studies of acupuncture treatment for allergic rhinitis could be undertaken to reveal whether this rate has been consistent across studies. Similarly the calculation of responder rates from previous studies of acupuncture for other conditions would be valuable.

8.4 Comparison

8.4.1 Success of subject blinding

The exit debrief questionnaire tested the credibility of the blinding procedure to the participants in the real and sham acupuncture groups after the last treatment in Week 8. Since the exit debrief questionnaire demonstrated that almost exactly half of both the real and sham acupuncture groups believed they were receiving real acupuncture, any effects of positive expectation (placebo) deriving from belief that the acupuncture was real, or negative expectation (nocebo) as a result of believing that the treatment received was sham, should be evenly distributed across both groups. Hence no differences between the groups can be adequately explained by either placebo or nocebo effects.

8.4.2 Real vs sham/placebo acupuncture

Five styles of sham acupuncture have been used in acupuncture studies to date, however there is mounting evidence that none of these sham protocols are inert (Dincer and Linde, 2003; Linde et al., 2010). This introduces a methodological problem into sham-controlled acupuncture trials, as the use of a sham protocol which is not inert introduces a negative bias into studies (Birch, 2006). For a sham-controlled trial to be valid there

are two essential requirements. Firstly, the sham protocol must be credible to the subject, so that blinding to sham or real treatment allocation can be achieved. The results of the exit debrief questionnaire in this study shows the sham protocol was credible since there was no difference between the real and sham groups in terms of who guessed they were receiving real treatment. Secondly, the sham protocol must be inert. In this study the sham protocol was clearly not inert, as subjects in the sham acupuncture group experienced some, though not all, of the benefits of those who received real acupuncture. Three tools were used to assess symptoms and quality of life, the iTNSS, the MiniRQLQ and the daily symptom and medication dairy. Results from both the iTNSS and daily symptom and medication dairy showed significant clinical benefits only in the real acupuncture group, and not in the sham acupuncture group. On the other hand, the Mini RQLQ showed significant benefits from both real and sham acupuncture in total scores and in all five domain scores. At Week 9 there was very little difference between the real acupuncture and sham acupuncture scores on the MiniRQLQ. However at Week 12 (four-week follow-up) the real acupuncture group were continuing to improve, in contrast to the sham acupuncture group whose condition had deteriorated since Week 9 (see Chapter 4.5.2).

From these results it can be concluded that the sham acupuncture protocol used in this study was credible, but not inert. It is also clear that there were differences between the response to real acupuncture and the response to sham acupuncture, both in terms of benefits that occurred exclusively in the real acupuncture group, and also in terms of the longevity of improvements which occurred only after real acupuncture.

8.5 Outcomes

(i) Primary Outcome

Modulation of the mucosal immune response in the upper respiratory tract by acupuncture in subjects with allergic rhinitis.

(ii) Secondary Outcome

A reduction in markers of mucosal inflammation (nasal airway resistance, symptoms and ECP) and improved quality of life scores following acupuncture.

8.5.1 Interpretation of the laboratory outcomes

In this study total IgE and allergen-specific IgE for house dust mite were significantly decreased at four-week follow-up, but allergen-specific IgEs for three regionally relevant grass pollens were unchanged. Statistically significant down-regulation of SP was seen after treatment in Week 1 and Week 3, but was not significant at Week 6 and Week 12. There were no significant differences between time-points for VIP, although there was a significant difference between real and sham acupuncture groups at Week 1 (18-24 hours post-treatment) ($p = 0.041$*). It is unclear whether this between-group difference has any clinical significance. No changes were seen in CGRP, NGF, BDNF, ECP or cytokines.

Hence, some modulation of the mucosal immune response in the upper respiratory tract was demonstrated in adults following acupuncture treatment for persistent allergic rhinitis. Statistically significant down-regulation of both immunoglobulins and pro-inflammatory neuropeptides was observed.

After acupuncture treatment, total IgE decreased by 17.9% ($p = 0.016$*) while allergen-specific IgE for house dust mite decreased by 24.0% ($p = 0.035$*). One previous study on acupuncture for persistent allergic rhinitis reported a post-treatment decrease in total IgE of 21.2% ($p<0.01$*) (Rao and Han, 2006). This statistically significant reduction in IgE is also likely to be clinically significant.

Statistically significant down-regulation of SP was seen 18 to 24 hours after real acupuncture treatment in Week 1 and Week 3. Compared to pre-treatment at Week 1, at 18 to 24 hours post-treatment in Week 1, SP had decreased by 83.2%, and at 18 to 24 hours post-treatment in Week 3, SP had decreased by 85.5%. In Week 6 at 18 to 24 hours post-treatment, a decrease in SP of 81.5% was still evident and at Week 12, a decrease of 42.1% was seen, however these differences were not statistically significant (see Table 1). After electroacupuncture for persistent allergic rhinitis, Li et al reported a decrease of 21.7% in SP and 33% in VIP immediately after treatment (Li et al., 2007b). Compared to pre-treatment at Week 1, at 18 to 24 hours post-treatment in Week 1, VIP had decreased by 27.4%, and at 18 to 24 hours post-treatment in Week 3, VIP had decreased by 40.4%. In Week 6 at 18 to 24 hours post-treatment, there was a decrease in VIP of 45.8% and at Week 12, a decrease of 42.0% was seen, however these

differences were not statistically significant (see Table 2). Compared to Li's study, the SP changes in this study were 4 times greater immediately after treatment, but VIP changes were of a similar order..

Table 1. Comparison between this study and one previous study on SP levels after acupuncture in persistent allergic rhinitis

SP in saliva McDonald et al 2014					SP in plasma Li et al 2007				
Week	n	Mean (±SE) (pg/ml)	% difference	P value (2-tailed)	Time	n	Mean (±SE) (pg/L)	% difference	P value (2-tailed)
1 pre	34	432.19±316.5			pre	50	58.62±35.13		
1 (18-24 hrs)	34	90.78±22.5	83.2%	0.039*	post	50	45.88±23.25*	21.7%	<0.05
3 (18-24 hrs)	33	74.19±16.8	85.5%	0.041*					
6 (18-24 hrs)	33	110.42±29.9	81.5%	0.256					
12	36	240.7±114.9	42.1%	0.475					

Abbreviations: n – number of subjects, SE – standard error, pg/ml – picograms per millilitre, pg/L – picograms per litre [NB: this appears to be a typographical error for pg/ml]

* p <0.05

Table 2. Comparison between this study and one previous study on VIP levels after acupuncture in persistent allergic rhinitis

VIP in saliva McDonald et al 2014					VIP in plasma Li et al 2007				
Week	n	Mean (±SE) (pg/ml)	% difference	P value (2-tailed)	Time	n	Mean (±SE) (pg/L)	% difference	P value (2-tailed)
1 pre	31	53.12±26.9			pre	50	56.86±30.41		
1 (18-24 hrs)	31	38.57±15.3	27.4%	0.620	post	50	38.07±17.15**	33.0%	<0.01
3 (18-24 hrs)	32	31.61±9.7	40.4%	0.460					
6 (18-24 hrs)	32	28.49±5.8	45.8%	0.384					
12	34	29.37±7.2	42.0%	0.421					

Abbreviations: n – number of subjects, VIP – vaso-active intestinal peptide, SE – standard error, pg/ml – picograms per millilitre., pg/L – picograms per litre [NB: this appears to be a typographical error for pg/ml]

* p <0.05, **p<0.01

There were no significant correlations between symptom reductions reported in daily symptom diaries for Weeks 1,3,6 and 12 and SP levels in saliva at 18 to 24 hours post-treatment in Weeks 1, 3 and 6 and in Week 12 (p = 0.474, 0.232, 0.316 and 0.547 respectively on Spearman's rho). One notable difference was that SP levels were rising between Week 6 and Week 12, while symptom scores were falling. Hence improvements in symptoms seen in the real acupuncture group in this study cannot be attributed solely to changes in SP. Also it is interesting to note that values for VIP, although not statistically significant, appeared to still be dropping in Week 6 when SP

was already beginning to rise again, and in Week 12 had only begun to rise again very slightly. This may suggest that modulation of SP by acupuncture is faster to induce and faster to decay than modulation of VIP. In both studies variability for both SP and VIP tended to rise and fall in tandem with the rise and fall of overall values.

8.5.1.1 How does acupuncture result in down-regulation of SP?

[for details of the effects of acupuncture on TRPV1 receptor and its pathways, see Appendix A]

One possibility is that acupuncture reduces the expression and sensitivity of TRPV1 receptor. Since TRPV1 receptor activation mediates the production and exocytotic release of SP and CGRP from sensory neurons (such as sensory C fibres in the upper layers of the nasal epithelium), any reduction in TRPV1 activation would reduce the release of SP.

The high affinity NGF receptor trkA can generate production and release of SP, hence any down-regulation of NGF could also reduce SP production and release. In this study no changes in NGF were seen, however no data on NGF levels were available at the same time-points when SP down-regulation was observed. So while no link between NGF and SP down-regulation was demonstrated, it also cannot be ruled out as a possible pathway.

In an inflammatory pain model, acupuncture has been shown to be capable of blocking the phosphatidylinositol 3-kinase/phosphatidylinositol phosphate 3 (PI3K/PIP3) signalling pathway between the trkA receptor and TRPV1 receptor, hence preventing any NGF-induced increase in the sensitivity and expression of TRPV1.

It has been reported that SP and CGRP inter-promote each other and also act synergistically in inflammatory oedema, so any down-regulation in CGRP might also lead to reduced production and release of SP. Like NGF, time-point data on CGRP levels at the same time-points when SP was significantly decreased were not captured in this study, so this question remains unanswered.

Adenosine may be involved in acupuncture-induced inhibition of TRPV1. Acupuncture has been demonstrated to trigger the release of adenosine, which, in turn, has been

shown to inhibit the activation of TRPV1 (Goldman et al., 2010; Tominaga and Tominaga, 2005).

Finally, acupuncture can trigger the production and release of the opioid neuropeptide met-enkephalin, which has been shown to directly antagonise SP production and release (Ai, 1986; Kondo, 2005; Yonehara et al., 1992).

Further research is required to elucidate the precise pathways via which acupuncture down-regulates Substance P, the time-course of this effect, and the clinical significance of down-regulation of SP in improving clinical symptoms of allergic rhinitis.

8.5.2 Interpretation of the clinical outcomes

The secondary outcome for this study was to investigate whether or not there was a reduction in markers of mucosal inflammation (nasal airway resistance, symptoms and ECP) and improved quality of life scores following acupuncture.

Statistically significant improvements in symptoms and quality of life, and reduced use of relief medication were observed after acupuncture. However, no statistically significant reductions in nasal airway resistance (peak nasal inspiratory flow or mean inferior turbinate obstruction) or ECP were seen. In a previous study on acupuncture for allergic rhinitis, nasal patency (measured with acoustic rhinometry) improved at 7.5 minutes after a single acupuncture treatment, but by 15 minutes differences were no longer statistically significant (Park, 2005b). In this study nasal patency was measured at one week and four weeks after acupuncture, so it is possible that any changes which might have occurred in nasal patency escaped detection due to these changes being very short-lived.

According to the daily symptom diary records, statistically significant improvement was first seen in the third week of treatment and persisted until four weeks after the last treatment. All three subjective measures (iTNSS, MiniRQLQ and daily symptom diary) showed statistically significant improvements in symptoms and quality of life in the real acupuncture group.

In the sham acupuncture group, there were significant improvements shown on the MiniRQLQ, but these improvements were not seen on the iTNSS and the daily

symptom diary. On the MiniRQLQ total scores and also on all five MiniRQLQ domain scores there was a consistent pattern of continuing improvement from Week 9 to Week 12 in the real acupuncture group, but of deterioration from Week 9 to Week 12 in the sham acupuncture group.

8.5.3 Changes in symptoms and quality of life scores in studies on acupuncture for allergic rhinitis

In studies on acupuncture treatment for allergic rhinitis to date, numerous different outcome measures have been used to evaluate changes in symptoms and quality of life. To facilitate comparisons, all symptom and quality of life scores have been converted to percentage change from baseline figures (see Table 7).

Generally, studies to date have consistently shown both statistically and clinically significant improvements in symptoms and quality of life immediately after acupuncture treatment with nasal symptom improvements ranging from 20.2% to 66.5%, and RQLQ scores improving between 37.0% and 51.6% (see Table 7). Symptoms and quality of life continued to improve during the follow-up period at 4 weeks, 8 weeks and 3 months compared to immediately post-treatment and at 1 week follow-up. However at 6 months follow-up, scores had deteriorated from the 3 month follow-up levels (see Table 7). Due to only two studies being currently available on intermittent allergic rhinitis (IAR/SAR) and only one of these with follow-up data, it is not possible to determine whether a similar pattern of changes in symptoms over time occurs in IAR/SAR studies (Brinkhaus et al., 2013; Xue et al., 2002). It has already been noted that improvements in symptoms and quality of life in response to sham acupuncture do not demonstrate this longevity. In fact at four-week follow-up in this study, MiniRQLQ scores were higher (that is symptoms were worse) than at one-week follow-up in the sham acupuncture group, but were lower in the real acupuncture group (see Chapter 4.5.2).

Table 3. Comparison of changes in symptoms and quality of life scores in studies on acupuncture for allergic rhinitis

Author and year	Outcome measure	Percentage change from baseline with p values				
		post-treatment	1 week f/u	4 week f/u	8 week f/u	3 – 6 mths f/u
Xue et al, 2002 (SAR)	TNSS	66.5% p = 0.003*				
	TNNSS	75.8% p = 0.032*				
Ng et al, 2004 (PAR in children)						**f/u (3-6 mths)**
	Daily rhinitis scores	20.2% p = 0.07				17.5% p = 0.03*
	Symptom free days	247.0% p=0.0001**				293.2% p = 0.0001**
	RMS	23.3% p = 0.661				3.3% p = 986
Rao et al, 2006 (PAR)						**6 mths f/u**
	AR symptom score	59.5% p < 0.001**				37.7% p < 0.001**
Xue et al, 2007 (PAR)						**3 mths f/u**
	TNSS	36.3% p = 0.01*				44.4% p = 0.001**
	RMS	43.1% p = 0.001**				36.1% p = 0.02*
Brinkhaus et al, 2008 (AR –type not specified)	RQLQ	51.6% p <0.001**				
	SF-36 physical	5.8% p <0.001**				
	SF-36 mental	5.6% p <0.001**				
Choi et al, 2013 (PAR)	TNSS	36.4% p = 0.029*	37.0% p = 0.099	42.2% p = 0.044*		
	TNNSS	29.8% p = 0.563	29.4% p = 0.751	51.3% p = 0.563		
	RQLQ	37.4% p = 0.073		40.8% p = 0.235		
Brinkhaus et al, 2013 (SAR)	RQLQ	37.0%			59.3%	
	RMS	65.2%			87.0%	
	VAS score	44.6%			69.0%	
	SF-36 physical	4.2%			7.0%	
	SF-36 mental	6.0%			5.9%	
McDonald et al 2014 (PAR)	iTNSS		28.2% p = 0.010*	34.3% p = 0.003*		
	MinRQLQ		23.2% p= 0.001**	30.7% p = 0.001*		
	Daily symptom dairy	22.3% p= 0.001**	19.9% p = 0.009*	24.3% p = 0.003*		

Abbreviations: f/u – follow-up, mths – months, SAR – seasonal allergic rhinitis, PAR – persistent allergic rhinitis, TNSS – total nasal symptom scores, TNNSS, total non-nasal symptom scores, RMS - relief medication scores, AR – allergic rhinitis, RQLQ – rhinitis quality of life questionnaire, SF-36 – short form 36 questionnaire, VAS – visual analog scale, iTNSS – instantaneous total nasal symptom score, MinRQLQ – Mini rhinoconjunctivitis quality of life questionnaire

In attempting to understand the immunological changes which might underpin the improvements in clinical symptoms and quality of life following acupuncture treatment for allergic rhinitis, it is necessary to examine both very short-term modulations of the mucosal immunity (hours to days), and also medium-term modulations (weeks to months). An 83.2% decrease in Substance P (SP) was observed in this study 18 to 24 hours after the first acupuncture treatment. This is both statistically and clinically significant. In Weeks 3 and 6, SP was 85.5% and 81.5% below baseline respectively, which was also clinically significant, however although the Week 3 data were statistically significant, the Week 6 data were not. The first indication that symptoms were improving significantly was seen in the daily symptom diaries in Week 3, hence the Substance P down-regulation preceded the symptomatic improvement by two weeks. By Week 12 (four-week follow-up), SP was only 42.1% below baseline and no longer statistically significant, however symptoms and quality of life measures were continuing to improve. From these data it can be concluded that although short-term down-regulation of Substance P may play a role in the early modulation of the mucosal immunity during acupuncture treatment of allergic rhinitis, that there is no direct connection between the levels of Substance P and the changes which occur in symptoms and quality of life measures. It seems unlikely that Substance P has any significant role in the medium-term modulation of mucosal immunity in allergic rhinitis.

Correlations between PNIF and mean turbinate obstruction, and between objective measures of nasal patency and subjective self-reporting of sensations of nasal obstruction have been discussed in Chapter 4.6.1. Similarly, a lack of positive correlation was noted between daily symptom scores and daily relief medication use, suggesting that self-medication behaviours may be influenced by factors other than symptom severity. For example, it is possible that some participants regarded relief medication as prophylaxis, and so decided to take medication regularly even in the absence of symptoms. This observation does highlight the need to take relief medication use measurements in context with other outcome measures, when designing and interpreting studies.

8.6 Conclusions

The primary outcome for this study was to determine whether or not there was any

modulation of the mucosal immune response in the upper respiratory tract by acupuncture in subjects with allergic rhinitis.

Statistically significant decreases were observed in total IgE and allergen-specific IgE for house dust mite at four-week follow-up, but allergen-specific IgEs for three local grass pollens were unchanged. Decreases in total IgE (17.9%) and allergen-specific IgE for house dust mite (24.0%) were likely to be clinically significant, especially the reduction in house dust mite IgE, which plays a pivotal role in the priming of mast cells in persistent allergic rhinitis. A significant reduction in total and house dust mite IgE also provides evidence of a shift in Th1/Th2 balance, even though this was not reflected in direct measurements of Th1 and Th2 cytokine levels at four-weeks follow-up (see Figure 1).

Statistically significant down-regulation of SP was seen 18 to 24 hours post-treatment in Week 1 and Week 3. Compared to pre-treatment at Week 1, at 18 to 24 hours post-treatment in Week 1, SP had decreased by 83.2%, and at 18 to 24 hours post-treatment in Week 3, SP had decreased by 85.5%. In Week 6 at 18 to 24 hours post-treatment, a decrease in SP of 81.5% was still evident and at Week 12, a decrease of 42.1% was seen, however these differences were not statistically significant. Down-regulation of SP and VIP after acupuncture treatment for allergic rhinitis is consistent with the results of one previous study (Li et al., 2007b). No changes were seen in CGRP, NGF, BDNF, ECP or cytokines.

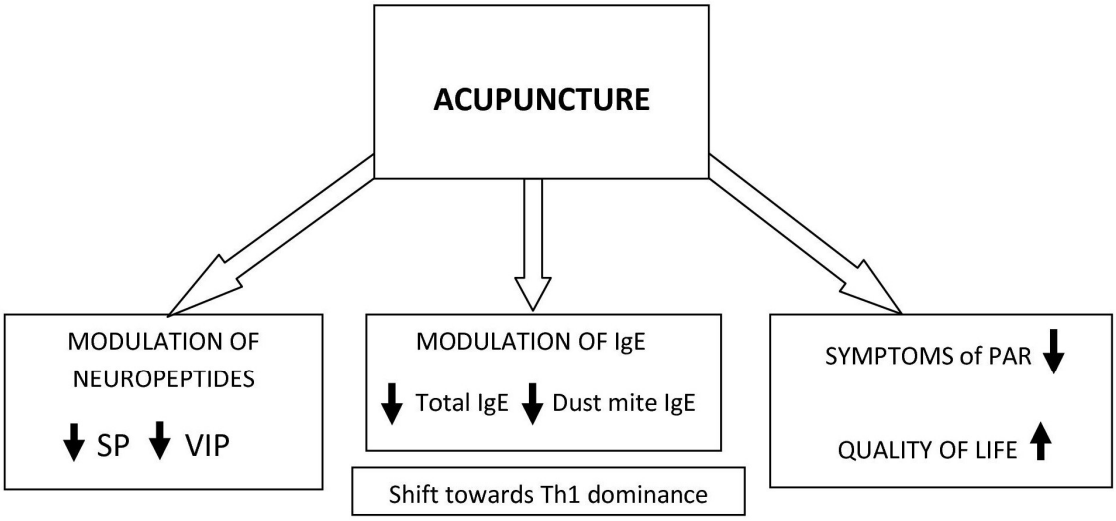
With regard to the secondary outcome for this study, statistically significant improvements in symptoms and quality of life, and reduced use of relief medication were observed after acupuncture. However, no statistically significant reductions in nasal airway resistance (peak nasal inspiratory flow or mean inferior turbinate obstruction) or ECP were seen. Since a previous study found that acupuncture-induced improvements in nasal airways begin to deteriorate 15 minutes after treatment, one week and four weeks follow-up were probably not appropriate time-points to capture any changes in nasal patency (Park, 2005b).

Real acupuncture in this study was effective in improving clinical symptoms and quality of life in adults with persistent allergic rhinitis (see Figure 1). With no serious adverse

events, and a minor adverse events rate of 3%, acupuncture was also shown to be a safe treatment.

Improvements in symptoms (as shown by the daily symptom diary) first appeared in the third week of treatment and persisted at four-week follow-up. The "responder rate" in the real acupuncture group was 86.1%.

Figure 1. Findings of this study on the effects of acupuncture on mucosal inflammation in PAR



Abbreviations: PAR – persistent allergic rhinitis, SP – substance P, VIP – vaso-active intestinal peptide, IgE – immunoglobulin E

The use of relief medication was significantly lower at four weeks follow-up (Week 12) than at baseline, but only in the real acupuncture group. However there was no correlation between use of relief medication and symptoms severity, suggesting that medication use was influenced by factors other than perception of symptoms.

Objective measurements of nasal patency did not correlate with subjective reports of perceived nasal obstruction. This is consistent with previous studies.

The sham acupuncture protocol used in this study was shown to be credible to subjects, but not inert. This introduces a negative bias into the study when attempts are made to interpret comparisons between the effects of real and sham acupuncture. Placebo and/or nocebo responses cannot adequately account for the effects of sham acupuncture in this study as the exit debrief questionnaire indicated that expectation and belief were almost identically distributed between the real and sham acupuncture groups.

8.7 Future directions in research

In this study it was planned to observe any short-term and medium-term changes which might occur in two neurotrophins, three pro-inflammatory neuropeptides and an array of Th1 and Th2 cytokines. In the case of Substance P (SP) and vaso-active intestinal peptide (VIP) this was achieved, and findings were broadly consistent with one previous study which also measured SP and VIP in adults with allergic rhinitis after acupuncture (Li et al., 2007b). However due to levels of CGRP, NGF and BDNF being below detection threshold in saliva, it was not possible to measure short-term changes, only changes at Week 12 for which plasma was used. It is possible that the reason why no changes were observed at Week 12 compared with Week 0, was that the effects of acupuncture on these substances was short-term (as was seen in SP) and hence not captured in this study.

In future studies, when investigating the short-term effects of acupuncture on modulation of mucosal immunity, it is recommended that sample collections for biomarkers focus more attention on time-points within the first 6 to 72 hours after acupuncture treatment (e.g.: pre-treatment, 6 to 8 hrs post-treatment, 18 to 24 hours post-treatment, 48 hours post-treatment, 72 hours post-treatment). In this study significant improvements in daily symptoms were seen in the third week of treatment, so when investigating short-term changes in neurotrophins, neuropeptides or cytokines, it may not be necessary to treat twice weekly for 8 weeks. A review of previous studies on acupuncture treatment for allergic rhinitis shows that statistically significant results occurred in all studies which used a treatment regimen of at least two treatments per week for at least 6 weeks (see Chapter 1.5.4). However this duration may not be necessary to begin to observe short-term changes in neurotrophins, neuropeptides or cytokines.

The possibility that acupuncture may interrupt signalling pathways to the transient potential vallinoid receptor (TRPV1) or may influence mast cell degranulation, as discussed in the review (Appendix A, section 5.10), are possible new directions in research to elucidate the anti-inflammatory effects of acupuncture in allergic rhinitis.

Whatever discoveries may be made in the future about the short-term effects of acupuncture on neuropeptides, neurotrophins and cytokines, the intriguing question which remains is how acupuncture can have lasting effects, specifically, what is the physiological process which accounts for ongoing improvements in symptoms for up to three months after treatment ceases? In research on acupuncture for pain relief, it has been suggested that while changes in opioid and non-opioid neurotransmitters may account for short-term changes, that to account for lasting changes in pain, some form of central neuromodulation (neuroplasticity) is likely to be involved (Warfield, 2004). Reversal of pathological neuroplasticity in the somatosensory cortex (in carpal tunnel syndrome pain) after acupuncture has already been demonstrated with functional magnetic resonance imaging (fMRI) (Napadow et al., 2006) . Perhaps some form of medium-term immunomodulation underpins lasting changes in symptoms of allergic rhinitis following acupuncture.

Further research into the anti-inflammatory effects of acupuncture, both in allergic rhinitis and in other clinical contexts, may also help to clarify the nature of any lasting immunomodulation which may be involved.

APPENDICES

Appendix A: McDonald, J.L., Cripps, A.W., Smith, P.K., Smith, C.A., Xue, C.C., and Golianu, B. (2013): The anti-inflammatory effects of acupuncture and their relevance to allergic rhinitis: A narrative review and proposed model. Evidence-Based Complementary and Alternative Medicine Article ID 591976. (12 pages)

Appendix B: Conference abstracts

Appendix C: iTNSS (instantaneous total nasal symptom score) (1 page)

Appendix D: Mini Rhinoconjunctivitis Quality Of Life Questionnaire (MiniRQLQ) (3 pages)

Appendix E: Daily Symptom and Medication Record Sheet (1 page)

APPENDIX A

McDonald, J.L., Cripps, A.W., Smith, P.K., Smith, C.A., Xue, C.C., and Golianu, B. (2013): The anti-inflammatory effects of acupuncture and their relevance to allergic rhinitis: A narrative review and proposed model. Evidence-Based Complementary and Alternative Medicine Article ID 591976.

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Review Article

The Anti-Inflammatory Effects of Acupuncture and Their Relevance to Allergic Rhinitis: A Narrative Review and Proposed Model

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Classical literature indicates that acupuncture has been used for millennia to treat numerous inflammatory conditions, including allergic rhinitis. Recent research has examined some of the mechanisms underpinning acupuncture's anti-inflammatory effects which include mediation by sympathetic and parasympathetic pathways. The hypothalamus-pituitary-adrenal (HPA) axis has been reported to mediate the antioedema effects of acupuncture, but not antihyperalgesic actions during inflammation. Other reported anti-inflammatory effects of acupuncture include an antihistamine action and downregulation of proinflammatory cytokines (such as TNF-α, IL-1β, IL-6, and IL-10), proinflammatory neuropeptides (such as SP, CGRP, and VIP), and neurotrophins (such as NGF and BDNF) which can enhance and prolong inflammatory response. Acupuncture has been reported to suppress the expression of COX-1, COX-2, and iNOS during experimentally induced inflammation. Downregulation of the expression and sensitivity of the transient receptor potential vallinoid 1 (TRPV1) after acupuncture has been reported. In summary, acupuncture may exert anti-inflammatory effects through a complex neuro-endocrino-immunological network of actions. Many of these generic anti-inflammatory effects of acupuncture are of direct relevance to allergic rhinitis; however, more research is needed to elucidate specifically how immune mechanisms might be modulated by acupuncture in allergic rhinitis, and to this end a proposed model is offered to guide further research.

1. Introduction

Worldwide, allergic rhinitis is estimated to affect 18% of 15–34-year olds and 10% of 35–54-year olds [1]. Studies estimate that seasonal allergic rhinitis affects approximately from 10% to 20% of the general population of the United States of America (USA), with an even greater prevalence in children [2, 3]. An estimated 30 to 60 million people annually in the USA suffer from allergic rhinitis [2].

While the term "rhinitis" implies inflammation of nasal mucus membranes, clinically rhinitis can refer to any nasal disorder which includes any one or more of the symptoms: sneezing, nasal pruritus, rhinorrhea, and nasal congestion [2]. Rhinitis can be allergic (triggered by contact with an inhaled allergen) or nonallergic [4]. Allergic rhinitis is the most common form of chronic rhinitis, however, up to 87% of patients with allergic rhinitis also react to triggers which are not allergens such as cold air, perfumes, and smoke [2].

Links between allergic rhinitis and asthma have been highlighted by the allergic rhinitis and its impact on asthma (ARIA) group which recommends that allergic rhinitis and asthma should be regarded as related phenomena of airway reactivity and managed using a "united airway approach" [3, 5, 6].

While there is evidence that acupuncture treatment is clinically beneficial for patients with allergic rhinitis, little is currently understood about the mechanisms of acupuncture in this, or other chronic inflammatory diseases which involve changes in either the systemic or mucosal immune response. This paper will address the current state of research into the effects of acupuncture on the immune system with emphasis on anti-inflammatory actions, and specifically on effects on mucosal immunity in allergic rhinitis. Based on this paper, a model that hypothesizes the potential anti-inflammatory mechanisms of acupuncture for allergic rhinitis is proposed to guide future investigation.

2. Search Strategy

Two searches were conducted. The first search investigated the pathophysiology of allergic rhinitis with an emphasis on the roles of cytokines, proinflammatory neuropeptides, and neurotrophins. The second search identified the acupuncture research on allergic rhinitis and the anti-inflammatory actions of acupuncture, especially in allergic inflammatory response. Database searches were conducted using Medline, PubMed, ScienceDirect, EbscoHost, Wiley Online library, Cochrane Database of Controlled Trials and the search terms "acupuncture," "allergic rhinitis," "inflammation," "anti-inflammatory," "neurotrophin," "neuropeptide," "cytokine," "substance P," "SP," "calcitonin gene-related peptide," "CGRP," "vaso-active intestinal peptide," "VIP," "histamine," "TRPV1." In addition the following journals were hand searched: Acupuncture Research (Zhen Ci Yan Jiu) (1984–2010), World Journal of Acupuncture-Moxibustion (1992–2011), Journal of Traditional Chinese Medicine (English edition) (1981–2011), Journal of Acupuncture and Tui Na Science (2010), and American Journal of Acupuncture (1973–1999).

3. Physiological and Immune Mechanisms of Allergic Rhinitis

Allergic rhinitis manifests as an allergic inflammatory response, an IgE-mediated reaction involving a complex interaction between inflammatory cells including eosinophils and mast cells, their released inflammatory cytokines, proinflammatory neuropeptides which promote vasodilation and plasma extravasation and neurotrophins which prolong survival of inflammatory cells and contribute to hypersensitivity [4]. Disruption of the integrity of the nasal epithelium through cleaving of tight junctions by protease activities (due to inflammation or airborne allergens) exposes sensory nerve endings, which enhances the neurogenic inflammatory response, especially the release of substance P (SP) and calcitonin gene-related peptide (CGRP) [4].

The early-phase allergic response in allergic rhinitis is triggered within minutes of allergen inhalation when IgE antibody, bound to mast cells, recognizes allergens and causes degranulation and release of inflammatory mediators such as histamine, tryptase, leukotrienes, prostaglandin D_2, and proinflammatory cytokines such as tumour necrosis factor alpha (TNFα) and interleukin 4 (IL-4) [4]. This early-phase response is generally characterized by sneezing, nasal itching, and rhinorrhoea [2, 4]. Sneezing and nasal itching have been shown to be neural responses mediated by histamine activating the histamine receptor H1R and the transient receptor potential vallinoid 1 (TRPV1) [7–10] (see Figure 1). Rhinorrhoea is primarily a glandular response involving nasal epithelial cells but also has neural and vascular contributions [7, 11]. Plasma extravasation, and vasodilation caused by mediators such as leukotrienes, prostaglandin D_2, nitric oxide, and proinflammatory neuropeptides such as SP, CGRP, and vasoactive intestinal peptide (VIP) create nasal congestion [4, 12]. Kaise et al. found that, in guinea pigs, SP and CGRP released from nasal sensory nerves, possibly stimulated by mast cell-derived histamine, partially mediate the early-phase response [13]. Neurotrophin nerve growth factor (NGF) has been shown to contribute to early-phase response in allergic airway response but not to late-phase response in rats with allergic asthma [14].

Late-phase allergic response occurs from 4 to 8 hours after the initial early-phase response as cytokines and other inflammatory mediators set off a cascade of events which promotes the expression of adhesion molecules (which in turn increase the adhesion of eosinophils to endothelial cells) and promotes infiltration by eosinophils, basophils, and neutrophils into the superficial lamina propria of the nasal mucosa [4]. The symptoms of late-phase response are similar to those of early-phase response but with a greater predominance of nasal congestion [4]. SP, CGRP, and neurokinin A (NKA) (and their respective receptors NK-1, CGRP1, and NK-2) are reportedly involved in the late-phase response in allergic nasal obstruction in guinea pigs [13].

The release of proinflammatory cytokines such as TNFα and IL-4 from degranulated mast cells promotes the differentiation of CD4+ T helper (Th) cells into Th2 phenotype. This Th2 response promotes the production of eosinophils and the phenotype switching of B lymphocytes leading to increased production of IgE and increased proliferation and activation of mast cells [15]. The weighting of Th1/Th2 balance towards Th2 characterizes the allergic response [15].

3.1. The Role of Neuropeptides in Airway Inflammation. Nonopioid proinflammatory neuropeptides contribute to neurogenic inflammation by promoting vasodilation and plasma extravasation, notably in the nasal mucosa in allergic rhinitis [11]. The nasal mucosa has dense networks containing the proinflammatory neuropeptides SP, VIP, and CGRP which can arise from sensory and autonomic nerve fibres and from neuroendocrine cells found widely in the nasal mucosa [16]. SP has also been shown to be colocalized with TRPV1 and the neurotrophin receptor tyrosine kinase A (trkA) in airway-specific murine dorsal root ganglionic neurons [17]. In cultured rat trigeminal ganglionic neurons SP and CGRP were found to be colocalized with TRPV1, and also with three SNARE complex proteins: synaptobrevin 1, syntaxin 1

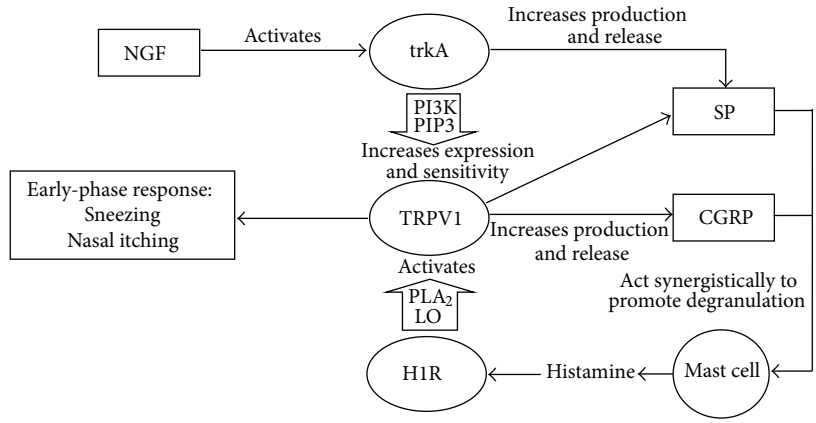

Figure 1: The role of transient receptor potential vallinoid 1 (TRPV1) in early-phase response in allergic rhinitis. Nerve growth factor (NGF) activates tyrosine kinase A (TrkA) receptor which in turn increases production and release of substance P (SP). Activation of TrkA receptor also initiates signalling via the PI3K/PIP3 pathway to increase expression and sensitivity of transient receptor potential vallinoid (TRPV1) receptor. TRPV1 receptor increases production and release of proinflammatory neuropeptides SP and CGRP which act synergistically to promote degranulation of primed mast cells. Histamine released by mast cells activates Histamine 1 receptor (H1R) producing signalling via the phospholipase A_2/lipoxygenase pathway to activate TRPV1, triggering early-phase allergic inflammatory response. NGF: nerve growth factor, TRPV1: transient receptor potential vallinoid 1, TrkA: tyrosine kinase A receptor, H1R: histamine 1 receptor, SP: substance P, CGRP: calcitonin gene-related peptide, PI3K/PIP3: phosphatidylinositol 3 kinase/phosphatidylinositol phosphate 3 pathway, PLA_2/LO: phospholipase A_2/lipoxygenase pathway.

Table 1

Role of SP and CGRP in allergic rhinitis:
(i) promote vasodilation and plasma extravasation in nasal epithelium (nasal congestion)
(ii) SP and CGRP act synergistically and potentiate each other in mast cell degranulation (early-phase allergic response) and plasma extravasation (nasal congestion)
(iii) activate monocytes to release pro-inflammatory cytokines (early-phase allergic response)
(iv) increase eosinophil accumulation in nasal mucosa during repeated allergen exposure
(v) SP promotes production and release of NGF

and SNAP 25, which mediate the exocytosis of CGRP from sensory neurons [18]. TRPV1 receptor activation mediates the production and exocytotic release of SP and CGRP from sensory neurons [17, 19].

In allergic airway inflammation (including allergic asthma and allergic rhinitis) SP and CGRP levels in the saliva and nasal secretions are elevated [16, 20]. SP in the nasal mucosa of humans increases eosinophil accumulation during repeated allergen exposure in allergic rhinitis [21]. SP and CGRP both activate monocytes to release the proinflammatory cytokines: TNF-α, IL-1β, IL-6, and IL-10 [22, 23]. The manner in which neuropeptides such as SP are able to modulate B-cell function is dependent on the activation of T cells by immunoregulatory cytokines such as IL-5 and TGFβ [24]. SP has also been reported to upregulate the expression of macrophage inflammatory protein 1β (MIP-1β) in human T lymphocytes (*in vitro*) [25]. SP and CGRP often act synergistically and also potentiate each other in inflammatory oedema, in plasma extravasation during airway inflammation and in mast cell degranulation [26–28] (see Table 1). NGF activation of the high-affinity NGF receptor trkA can generate production and release of SP, while SP, in turn, can promote the production and release of NGF [17, 20, 29]. NGF can also promote CGRP content and release from TRPV1-expressing trigeminal ganglion neurons *in vitro* [30] (Figure 1).

From this evidence it can be seen that proinflammatory neuropeptides such as SP, CGRP, and VIP interact with various immune cells including T lymphocytes, B lymphocytes, macrophages, monocytes, and mast cells to modulate allergic inflammation of the nasal mucosa. These interactions influence the release of cytokines and are capable of modifying Th1/Th2 balance in CD4+ T-cell differentiation. Proinflammatory neuropeptides can act synergistically and potentiate each other. Proinflammatory neuropeptides and neurotrophins can promote each other's production and release, creating a positive feedback cycle (Figure 2).

3.2. The Role of Neurotrophins in Airway Inflammation. Neurotrophins, or nerve growth factors, are proteins which regulate the survival, death, or differentiation of neurons. The primary function of neurotrophins is to promote nerve growth. The main categories of neurotrophins include NGF, brain-derived neurotrophic factor (BDNF), glial cell-derived

Table 2

Role of NGF in allergic rhinitis:
(i) increases neuronal abundance in nasal epithelium leading to hypersensitivity and increased tendency to nasal congestion
(ii) increases expression and sensitivity of TRPV1 receptors in nasal epithelium
(iii) prolongs survival of eosinophils and mast cells (prolonging inflammatory response)
(iv) contributes to early-phase allergic response (but not to late-phase response)
(v) increases production and release of pro-inflammatory neuropeptides SP and CGRP

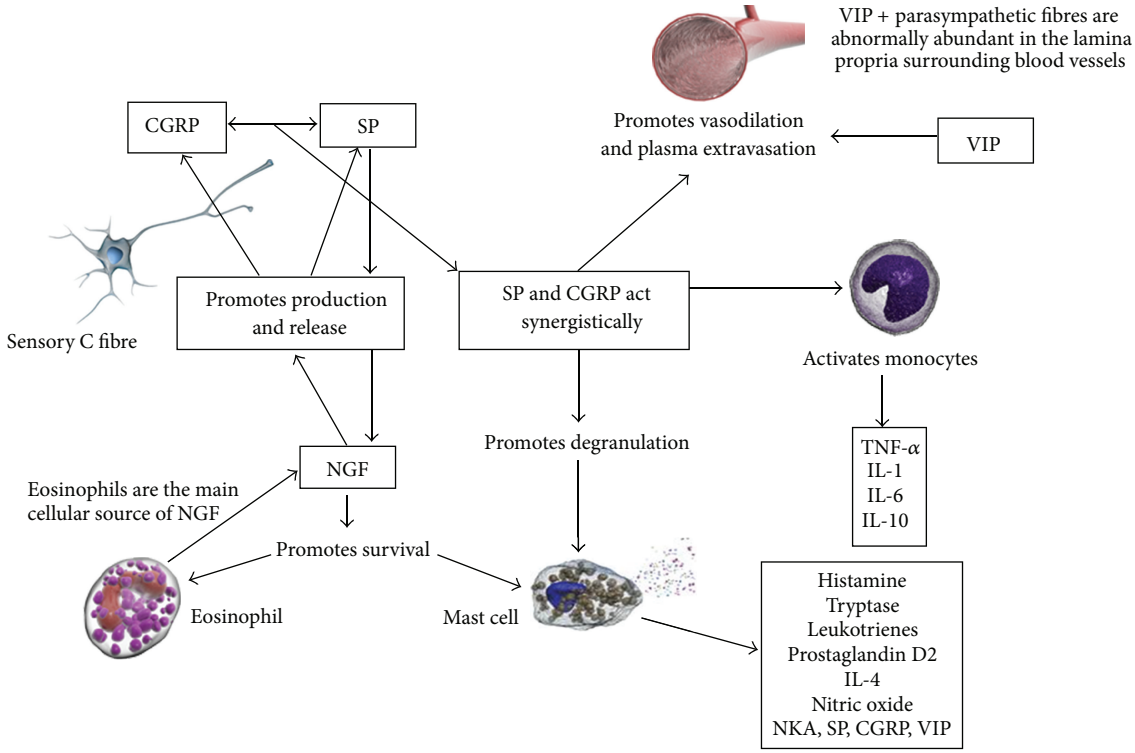

Figure 2: Complex crosstalk between inflammatory cells, neuropeptides, neurotrophins, and cytokines in allergic rhinitis. Substance P (SP) and calcitonin gene-related peptide (CGRP) act synergistically (along with vasoactive intestinal peptide (VIP)) to promote vasodilation and plasma extravasation causing nasal congestion. SP and CGRP also activate monocytes to release proinflammatory cytokines and promote degranulation of primed mast cells contributing to early-phase allergic response. Nerve growth factor (NGF) promotes the production and release of SP and CGRP and also promotes the survival of eosinophils and mast cells hence prolonging inflammatory response. CGRP: calcitonin gene-related peptide, SP: substance P, VIP: vasoactive intestinal peptide, NKA: neurokinin A, NGF: nerve growth factor, TNF-α: tumour necrosis factor alpha, IL-1: interleukin 1, IL-4: interleukin 4, IL-6: interleukin 6, IL-10: interleukin 10. Images courtesy of Dr P. K. Smith.

neurotrophic factor (GDNF), neurotrophin 3 (NT-3), and neurotrophin 4/5 (NT 4/5). The density of innervation to the nasal mucosa in allergic rhinitis patients is reported to be double that of healthy individuals [16, 31, 32]. Much of this additional innervation surrounds arterial blood vessels in the lamina propria and principally involves VIP-containing parasympathetic nerve fibres [32–34]. In allergic rhinitis this neuronal abundance is likely to contribute to hypersensitivity as well as to amplifying the allergic inflammatory response. Concentrations of NGF, BDNF, and NT-3 increase dramatically in the respiratory epithelium during allergic rhinitis [20, 35]. Both nasal NGF and BDNF expressions were reported to be significantly increased in allergic rhinitis patients compared to healthy controls after nasal allergen provocation [36]. This allergen-induced increase in BDNF correlated with the maximal increase in total nasal symptom score (TNSS), suggesting an important role for this neurotrophin in modifying symptom severity in allergic rhinitis patients [36].

In addition to promoting neuronal proliferation, neurotrophins also prolong the survival of eosinophils and mast cells, thereby prolonging the inflammatory response [20]. Eosinophils, mast cells, monocytes and macrophages, in turn, all produce NGF [20, 37, 38]. Wu et al. reported that the major sources of NGF in the human nasal mucosa are submucosal glands and nasal epithelium, with eosinophils being the major cellular source (while mast cells account for only a small fraction) [38] (Table 2).

These findings suggest not only an important role for SP, CGRP, and VIP in promoting and amplifying allergic airways inflammation, but also suggest a complex interaction

between inflammatory cells, cytokines, and neurotrophins with these proinflammatory neuropeptides (Figure 2).

3.3. The Role of TRPV1 Receptor in Early-Phase Allergic Inflammatory Response. TRPV1 receptor is a polymodal receptor which is activated by several triggers including capsaicin, noxious heat (42–53°C), low extracellular pH, ethanol, acids, pollution, protons, and lipids [17, 39]. TRPV1-positive cells are found on epithelial cells, vascular endothelial cells, submucosal glands, and nerves in human nasal mucosa [40]. TRPV1 has been shown to be colocalized with the neurotrophin receptor tyrosine kinase trk-A and SP in airway-specific murine dorsal root ganglionic neurons [17]. In cultured rat trigeminal ganglionic neurons, TRPV1 was found to be colocalized with SP, CGRP, and the SNARE complex proteins synaptobrevin 1, syntaxin 1 and SNAP 25 (which mediate the exocytosis of CGRP) [18]. The expression and sensitivity of TRPV1 receptor can be upregulated by NGF-induced activation of trkA receptor which signals via the PI3K/PIP3 pathway [41] (see Figure 1). TRPV1 increases the production and exocytotic release of proinflammatory neuropeptides SP and CGRP which act synergistically to promote the degranulation of primed mast cells [17].

Histamine is generally regarded as the archetypal mediator of allergic inflammatory response. Histamine released by degranulating mast cells activates the histamine 1 receptor (H1R) which in turn activates the TRPV1 receptor via the phospholipase A_2/lipoxygenase (PLA_2/LO) pathway [8]. Histamine-induced activation of TRPV1 triggers the early-phase response in allergic rhinitis [8]. These pathways are outlined in Figure 1.

4. Clinical Efficacy and Effectiveness of Acupuncture for the Treatment of Allergic Rhinitis

Two early systematic reviews concluded that there was insufficient evidence to demonstrate the efficacy of acupuncture in allergic rhinitis, however, these reviews were limited by the paucity and quality of studies available (3 and 7 studies up to 2004) [42, 43]. A more recent and more comprehensive systematic review (12 studies up to 2008 involving 1076 patients) concluded that acupuncture and moxibustion were safe and effective to treat allergic rhinitis and may have some advantages over routine medication [44]. Another recent systematic review (12 studies up to 2007) found suggestive evidence for the effectiveness of acupuncture in persistent allergic rhinitis, but no significant difference between real and sham acupuncture for seasonal allergic rhinitis [45]. This may be due to relatively few studies being published to date on seasonal allergic rhinitis.

Since publication of the most recent review, evidence of benefit has been further supported by findings from a large trial which included two randomized groups and one nonrandomized group, involving 5237 patients with allergic rhinitis. Brinkhaus and colleagues found that when acupuncture was added to routine medical care, there were statistically and clinically relevant benefits [46]. Rhinitis Quality of Life Questionnaire (RQLQ) scores after 3 months of acupuncture treatment improved by a mean (SE) of 1.48 (0.06) in the acupuncture group, by 0.50 (0.06) in the control group, with a difference in the improvement of 0.98 (0.08) ($P < 0.001$) [46].

5. Mechanisms by Which Acupuncture May Moderate the Clinical Symptoms of Allergic Rhinitis

5.1. Overview of Possible Anti-Inflammatory Mechanisms of Acupuncture. Recent research has elucidated some of the mechanisms underpinning acupuncture's anti-inflammatory effects. Multiple physiological pathways appear to mediate the anti-inflammatory effects of acupuncture including the hypothalamus-pituitary-adrenal (HPA) axis [47–50], sympathetic pathways (via both sympathetic postganglionic neurons and the sympathoadrenal medullary axis) [49, 50], and possibly parasympathetic cholinergic pathways [51–54].

Other relevant anti-inflammatory effects of acupuncture include antihistamine effects [55–58] and downregulation of proinflammatory cytokines (such as TNF-α, IL-1β, IL-6, and IL-10) [59–65], and proinflammatory neuropeptides (such as SP, CGRP, and VIP) [66, 67]. The involvement of both opioid and nonopioid neurotransmitters has been demonstrated [68–73]. Neurotrophins (such as NGF, BDNF, and NT-3) which contribute to hypersensitivity, as well as enhance and prolong inflammatory response, have been shown to be downregulated by acupuncture [74–78]. Acupuncture has also been found to suppress the expression of COX-1, COX-2, and iNOS during experimentally induced inflammation [79]. NMDA and AMPA/KA receptors (receptors for glutamate and aspartate) have also been implicated in the anti-inflammatory actions of acupuncture [80, 81]. The effects of acupuncture on TRPV1 have also been examined. Further research is needed to clarify the role these anti-inflammatory actions of acupuncture may play in the context of treatment for allergic rhinitis.

5.2. The HPA Axis. Acupuncture effects on the inflammatory response have been shown to be modulated by the HPA axis in a number of animal studies. In a carrageenan-induced paw inflammation rat model, the antioedema effects of electroacupuncture were abolished by various disruptions of the HPA axis including adrenalectomy and antagonizing receptors for corticotropin releasing hormone (CRH), adrenocorticotropic hormone (ACTH), or glucocorticoids [47, 48]. The involvement of the HPA axis in the anti-inflammatory effects of acupuncture was further supported by findings of significant increases in levels of ACTH and corticosterone in the same rat paw inflammation model in response to electroacupuncture [47, 48]. However, the disruption of the HPA axis has been found to have no effect on the antihyperalgesic effects of acupuncture or electroacupuncture suppression of leucocyte migration (in a mouse air pouch inflammation model) [47–49]. The HPA axis-mediated acupuncture inhibition of inflammatory oedema may be involved in the reduction of nasal congestion in allergic rhinitis.

Table 3: Th1/Th2 cytokines in studies of acupuncture for allergic rhinitis.

Reference	Measurement method	Th2 cytokines				Th1 cytokines	
		IL-1β	IL-4	IL-10	GM-CSF	IFN-γ	IL-2
[59]	Peripheral blood plasma			↓			↓
[60]	Peripheral blood serum		↓			No change	
[61]	RNA from peripheral blood	↓					
[62]	Supernatant from peripheral blood monocytes		↓		↓	↑	

Reported increase: ↑, Reported decrease: ↓.

5.3. Sympathetic Pathways. Leucocyte migration appears to be mediated by the activation of β-2 adrenoreceptors on leucocytes by noradrenalin released from sympathetic postganglionic neurons in response to low-frequency electroacupuncture [49, 50]. Low-frequency electroacupuncture leads to the suppression of carageenan-induced paw oedema in mice which appeared to be mediated via sympathetic postganglionic neurons [50]. Conversely, high-frequency electroacupuncture also had significant anti-inflammatory effects, this time mediated via the sympathoadrenal medullary axis [50]. Sympathetic mediation of acupuncture inhibition of inflammatory oedema may also be involved in alleviating nasal congestion in allergic rhinitis.

5.4. Parasympathetic Cholinergic Pathways. A parasympathetic anti-inflammatory pathway mediated by acetylcholine (ACh) has been demonstrated in research not related to acupuncture. ACh released by the vagus nerve binds to α7-nicotinic receptors (α7nAChR) on macrophages which inhibits the release of proinflammatory cytokines [51–54]. It has been proposed that this cholinergic anti-inflammatory pathway may be activated by acupuncture; however, no direct experimental confirmation of this hypothesis is currently available [51].

5.5. Antihistamine Action. Acupuncture has been reported to reduce histamine-induced itch in healthy subjects [55–57]. Prophylactic acupuncture (for 15 minutes prior to topical histamine application to the skin) was shown to significantly reduce histamine-induced itch and wheal formation in healthy subjects, compared with placebo-point acupuncture and no intervention [57]. Type I hypersensitivity itch, wheal, and flare response to allergen challenge in patients with atopic eczema was also significantly reduced by acupuncture [58].

A possible mechanism for an antihistamine action of acupuncture may be the downregulation of signalling in TRPV1 receptors, which mediate histamine-induced symptoms of allergic rhinitis such as nasal itching, sneezing, and rhinorrhoea [8, 10].

5.6. Cytokines. Changes in cytokines which would be expected to be associated with an improvement in allergic inflammation include downregulation in Th2 cytokines such as IL-4, IL-6, and IL-10 and proinflammatory cytokines such as IL-1, IL-6, and IL-10 accompanied by an upregulation in Th1 cytokines such as IL-2 and IFN-γ.

Some evidence of a shift in Th1/Th2 balance away from Th2 has been shown in studies of acupuncture treatment of allergic rhinitis in humans, namely, a significant reduction in IL-10 and IL-4 and a significant decrease in gene expression for IL-1R1 [59–61] (see Table 3). Rao and Han reported no change in IFN-γ in humans with allergic rhinitis; however, in another recent study, Zheng et al. did report a significant increase in IFN-γ together with a significant decrease in the Th2 cytokines IL-4 and GM-CSF (granulocyte-macrophage colony stimulating factor) [60, 62]. After two courses of 15-second-daily acupuncture treatments, IL-4 and GM-CSF decreased while IFN-γ increased ($P < 0.01$) until the levels of all three cytokines in peripheral blood were similar to those of the healthy controls [62].

Immediately after a single acupuncture treatment, Petti et al. reported a significant decrease in IL-10, no significant change in IL-6, and an unexpected significant decrease in IL-2 [59]. Since the effects of a single acupuncture treatment are not likely to accurately predict the effects of a substantial course of acupuncture, it is difficult to interpret the results of this study.

In other inflammatory conditions, studies measuring the effects of acupuncture on Th2 and proinflammatory cytokines have reported significant reductions in IL-1β and TNF-α in carrageenan-induced hind paw inflammation in rats [63]. Acupuncture has also been found to significantly reduce IL-6 and IL-10 in humans with asthma [64]. In a rodent model of experimental asthma, electroacupuncture increased IL-1 and IFNγ and decreased IL-4, IL-10, nitric oxide, and leukotriene B4 in bronchoalveolar lavage and pulmonary tissue compared with control and sham acupuncture groups [65]. Secretion of Th2 promoting cytokines IL-4 and IL-13 was suppressed after acupuncture in a study using DNP-KLH immunized mice [82].

5.7. Neuropeptides. Studies on the effects of acupuncture on neuropeptides can be divided broadly into research on opioid neuropeptides and nonopioid proinflammatory neuropeptides.

5.7.1. Opioid Neuropeptides. Much of the research on acupuncture suppression of inflammatory hyperalgesia overlaps with the broader research on acupuncture's antinociceptive actions and the role of opioid neuropeptides in these effects. Opioid neuropeptides which have been shown to mediate antinociceptive effects of acupuncture include enkephalins, β-endorphin, endomorphins,

Table 4: Summary of acupuncture effects on neurotrophins, IgE and eosinophils.

(a) Neurotrophins: biphasic action depending on disease and model

Disease	Model	Reported effect	Reference
Parkinson's disease	Rodent	Upregulates BDNF in substantia nigra	[86]
Retinitis pigmentosa	Rodent	Upregulates NGF and BDNF in retina	[87]
Spinal cord injury	Feline	Upregulates NGF in spine	[88]
Polycystic ovarian syndrome (PCOS)	Rodent	Downregulates NGF in ovaries	[74–76]

(b) Serum IgE: contradictory findings

Disease	Model	Reported effect	Reference
Allergic rhinitis	Human	No significant decrease	[60]
Allergic rhinitis	Human	No significant decrease	[89]
Allergic rhinitis	Human	No significant decrease	[90]
Allergic rhinitis	Human	Significant decrease	[91]

(c) Eosinophils: contradictory findings

Disease	Model	Reported effect	Reference
Allergic rhinitis	Human	No significant difference in nasal or blood eosinophils	[90]
Allergic rhinitis	Human	No significant difference in blood eosinophils	[92]
Allergic rhinitis	Human	Significant decrease in blood eosinophils and percentage of nasal eosinophils	[91]

dynorphins, and nociceptin/orphanin FQ, and different frequencies of electroacupuncture have been shown to stimulate the production and release of different neuropeptides in a highly selective manner [83].

In animal experiments, intraperitoneal injection of Naloxone, a generic opioid receptor antagonist, has been shown to significantly decrease electroacupuncture suppression of hyperalgesia and leucocyte migration [69, 84]. However electroacupuncture's effects in reducing oedema in a carageenan-induced hind paw inflammation model were unaffected by intraperitoneal Naloxone [68]. This finding is consistent with other studies which have shown that the antioedema effects of lowfrequency electroacupuncture are mainly mediated via the HPA axis and sympathetic postganglionic neurons, rather than via central or peripheral opioid pathways [47, 48, 50].

Another possible role for opioid neuropeptides in acupuncture for allergic rhinitis is the inhibition of non-opioid proinflammatory neuropeptides such as SP. The opioid neuropeptide enkephalin inhibits or regulates SP release from peripheral nerve endings via the activation of opiate receptors, suggesting a possible role for enkephalin in the downregulation of SP by acupuncture [67, 71, 72].

Opioid receptors have been identified on numerous types of immune cells including B-lymphocytes, T-lymphocytes, natural killer cells, granulocytes, and monocytes, however the role of endogenous opioid neuropeptides in the anti-inflammatory effects of acupuncture has yet to be elucidated [73].

5.7.2. Nonopioid Proinflammatory Neuropeptides (SP, CGRP, VIP). Only one study has examined the effects of acupuncture on the proinflammatory neuropeptides SP and VIP in humans with allergic rhinitis [66]. SP and VIP were measured in plasma from venous blood using radioimmunoassay. When one group receiving 30 treatments of electroacupuncture was compared to another group receiving medication (Cetirizine 10 mg three times daily), both groups showed a significant lowering of both SP and VIP after treatment compared to pretreatment [66]. The electroacupuncture group had a significantly greater reduction in VIP than the medication group, but there was no significant difference between groups in reduction of SP [66]. Decreased levels of SP and VIP were also closely correlated with improvements in clinical signs and symptoms [66].

The topical application of Chinese herbal paste to acupuncture points has also been reported to inhibit celiac mast cell degranulation in mice with allergic rhinitis [85]. These findings suggest that the inhibition of mast cell degranulation may be one of the important clinical effects of acupuncture in the early-phase response in allergic rhinitis and that this inhibition may be achieved, in part, by downregulation of proinflammatory neuropeptides such as SP, CGRP, and VIP (which have been shown to promote mast cell degranulation) [26–28].

5.8. Neurotrophins, IgE and Eosinophils. Acupuncture has been reported to upregulate and downregulate neurotrophins; however, to date there have been no studies measuring the effects of acupuncture in allergic rhinitis [77]. The reported effects of acupuncture on neurotrophins, IgE and eosinophils are summarized in Table 4.

5.9. Clinical Outcome Measurements: Nasal Congestion and Nasal Ciliary Clearance Rates. Acoustic rhinometry after a single acupuncture treatment for allergic rhinitis was used to measure nasal volume (cm^3) (NV) and total nasal minimal cross-sectional area (cm^2) (MCA) [93]. A statistically significant increase in NV and MCA was reported immediately after acupuncture in both active and placebo acupuncture

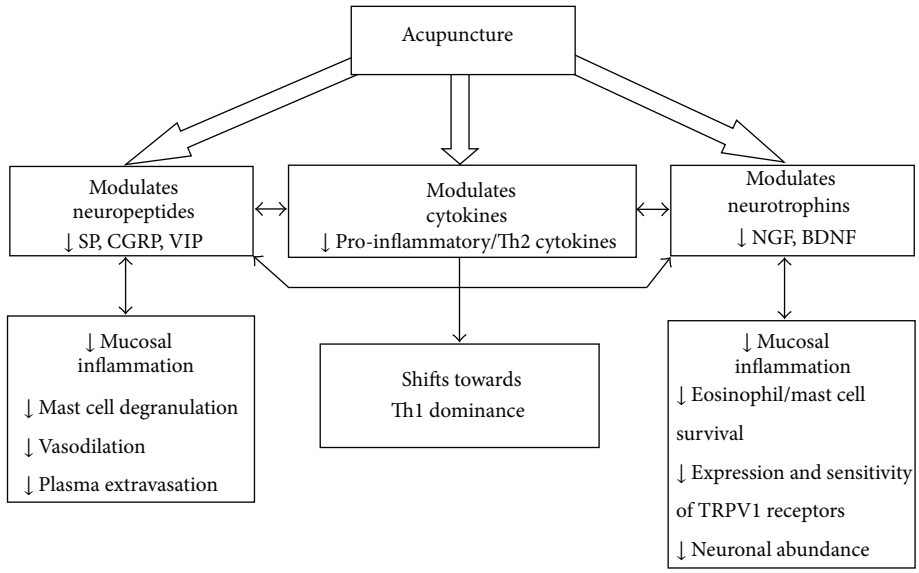

Figure 3: Proposed model for the effects of acupuncture in mucosal inflammation. CGRP: calcitonin gene-related peptide, SP: substance P, VIP: vasoactive intestinal peptide, NGF: nerve growth factor, BDNF: brain-derived neurotrophic factor, TRPV1: transient receptor potential vallinoid 1.

groups, with a greater increase in the active acupuncture group. In the active acupuncture group, increases in NV and MCA persisted for 15 minutes but were not significant after 7.5 minutes, while in the placebo group NV dropped below baseline after 7.5 minutes and MCA was also below baseline after 15 minutes. This suggests that a single acupuncture treatment has an immediate but very short-lived effect in decreasing nasal congestion in humans with allergic rhinitis.

In a prospective pragmatic open study involving 45 patients with allergic rhinitis, when acupuncture was compared to antihistamine medication over a seven-week period, nasal ciliary clearance rates increased significantly in both groups but were faster in the acupuncture group, both immediately after treatment and at 3-month followup [92].

5.10. The Effects of Acupuncture on TRPV1 Receptor in Early-Phase Allergic Inflammatory Response. Acupuncture has been shown to inhibit TRPV1 signalling, but the mechanism for this action remains unclear [94]. In a cancer pain model, electroacupuncture has been shown to suppress TRPV1 mRNA and protein upregulation in the dorsal root ganglia of tumour-bearing rats [94]. In an inflammatory pain model, the acupuncture-induced activation of A1 receptors by adenosine has been shown to be essential to the antinociceptive effects of manual acupuncture [95]. Adenosine can directly inhibit TRPV1 activation [96]. Another possible pathway is the downregulation of NGF activation of tyrosine kinase receptor trkA which uses the phosphatidylinositol 3-kinase/phosphatidylinositol phosphate 3 (PI3K/PIP3) signalling pathway to increase the expression and sensitivity of TRPV1 [41]. In an inflammatory pain model, electroacupuncture has recently been shown to inhibit phosphorylation of spinal PI3K, hence preventing the production of PIP3 and downstream protein kinase Akt [97]. This demonstrates that electroacupuncture is capable of blocking the PI3K/PIP3 signalling pathway which is essential to NGF-induced enhancement of TRPV1 expression and sensitivity.

In summary, acupuncture may inhibit TRPV1 expression and sensitivity by downregulating the production and release of NGF and/or by blocking PI3K/PIP3 signalling between trkA receptor and TRPV1. Acupuncture-induced inhibition of TRPV1 may be achieved by downregulating SP and CGRP which in turn would reduce degranulation of mast cells thereby reducing histamine release and histamine activation of TRPV1 via H1R. Another possibility is that downregulation of TRPV1 (regardless of the source of this downregulation) causes the reduction in SP and CGRP release, or perhaps this is a negative feedback loop. Acupuncture inhibition of TRPV1 may also involve adenosine release.

6. A Proposed Model for the Mechanism of Acupuncture in Allergic Rhinitis

Given the complex crosstalk between cytokines, neuropeptides and neurotrophins in allergic inflammation, it is hypothesized that acupuncture might exert anti-inflammatory actions in allergic rhinitis in three ways: firstly, by down-regulating Th2 and proinflammatory cytokines and up-regulating Th1 cytokines; secondly, by down-regulating proinflammatory neuropeptides (namely SP, VIP, and CGRP) and finally, by downregulating neurotrophins (NGF and BDNF) (see Figure 3).

If acupuncture can be shown to have these actions on modulating cytokines, neuropeptides, and neurotrophins in allergic rhinitis, then these modulations would be expected to be correlated with improvements in clinical signs and symptoms, including a reduction in hyperresponsiveness, sneezing, nasal itching, rhinorrhea, and nasal congestion.

Any reduction in Th2 dominance would also suggest a modulation of allergic status.

7. Conclusion

The role of neurotrophins in neurogenic inflammation, and in particular allergic airway inflammation, has recently been studied; however, the complex crosstalk between neurotrophins, neuropeptides, and cytokines in allergic airway inflammation is still poorly understood. Further studies are needed to elucidate some of the interactions between the neuropeptides: SP, CGRP, and VIP and the neurotrophins NGF and BDNF and some Th1, Th2, and proinflammatory cytokines in allergic rhinitis.

Acupuncture has been reported to improve clinical outcomes in patients with allergic rhinitis, and some aspects of the anti-inflammatory actions of acupuncture have been studied. Little research to date has investigated the mechanisms by which acupuncture may modulate immune response in the upper airways to improve clinical outcomes in patients with allergic rhinitis. Although the actions of some neuropeptides in the antinociceptive effects of acupuncture have been extensively studied, the possible contribution that both opioid and non-opioid neuropeptides may make to inflammation, specifically allergic inflammation, has yet to be clarified. It is suggested in our theoretical model that, in adult subjects with allergic rhinitis, acupuncture may down-regulate certain proinflammatory neuropeptides and neurotrophins as well as Th2 cytokines and proinflammatory cytokines, thereby producing a shift in the Th1/Th2 balance of T helper cells towards Th1. Together these hypothesized actions would be expected to alleviate clinical signs and symptoms of allergic rhinitis. Further studies, guided by this model, are needed (using both animal and human models) to explore the effects of acupuncture at the cellular level on the inflammatory cascade described above, as well as the clinical effects of this treatment on symptomatic relief over time.

Conflict of Interests

No author has declared a conflict of interests with respect to this study.

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APPENDIX B – Conference abstracts

AACMAC, Sydney, 2008 **- Keynote speaker**
McDonald, J. (2008) The Effects of Acupuncture on Immune Function. *Australasian Acupuncture and Chinese Medicine Annual Conference (AACMAC), Sydney*

AACMAC, Melbourne, 2009 **- Keynote speaker**
McDonald, J. (2009) The Anti-inflammatory Effects of Acupuncture. *Australasian Acupuncture and Chinese Medicine Annual Conference (AACMAC), Melbourne*

AACMAC, Adelaide, 2010 **- Invited speaker**
McDonald, J. (2010) The Effects of Acupuncture on Allergic Rhinitis. *Australasian Acupuncture and Chinese Medicine Annual Conference (AACMAC), Adelaide*

World Federation of Acupuncture-Moxibustion Societies (WFAS) 2010 International Acupuncture Conference in US, San Francisco
McDonald, J.L., Cripps, A.W., Smith, P.K., Smith, C.A., Xue, C.L. and Golianu, B. (2010) Acupuncture and Allergic Rhinitis: Efficacy and Mechanism. *WFAS International Acupuncture Symposium in US, San Francisco*

AACMAC, Perth, 2011 **- Invited speaker**
McDonald, J. (2011) Problems with Placebo, Sham and Minimal Acupuncture in Research. *Australasian Acupuncture and Chinese Medicine Annual Conference (AACMAC), Perth*

Gold Coast Health and Medical Research Conference, Seaworld Nara Resort, 2011 **– poster presentation**
McDonald, JL,. Cripps, AW, Smith, PK, Smith, CA, Xue, CL, Golianu, B
(2011) The Effects of Acupuncture on Mucosal Immunity in the Upper Respiratory Tract. *Gold Coast Health and Medical Research Conference, Seaworld Nara Resort*

AACMAC, Brisbane, 2012 **- Invited speaker**
McDonald, J. (2012) Future Directions for Acupuncture Research.
Australasian Acupuncture and Chinese Medicine Annual Conference (AACMAC), Brisbane

International Scientific Acupuncture and Meridian Symposium (iSAMS), 2012 **– Invited speaker**
McDonald, J.L. (2012) Acupuncture Research: Current Status and Future Directions. *International Scientific Acupuncture and Meridian Symposium (iSAMS),University of Technology Sydney (UTS), Sydney*

World Federation of Acupuncture-Moxibustion Societies 8th World Conference on Acupuncture, Sydney, 2013 **– Invited speaker**
McDonald, JL,. Cripps, AW, Smith, PK, Smith, CA, Xue, CL, Golianu, B
(2013) The effects of acupuncture on mucosal immunity in perennial allergic rhinitis: a randomised, subject-and-assessor-blinded, sham-controlled clinical trial. *World Federation of Acupuncture-Moxibustion Societies 8th World Conference on Acupuncture, Sydney*

AACMAC, Melbourne, 2014 **- Invited speaker – Best Research/Scientific Paper**
McDonald, JL,. Cripps, AW, Smith, PK, Smith, CA, Xue, CL, Golianu, B
(2014) The effects of acupuncture on mucosal immunity in persistent allergic rhinitis: a randomised, subject-and-assessor-blinded, sham-controlled clinical trial: final results. *Australasian Acupuncture and Chinese Medicine Annual Conference (AACMAC), Melbourne*

AACMAC SYDNEY 2008

The Effects of Acupuncture on Immune Function

John McDonald

School of Medicine, Griffith Health Institute, Griffith Health, Griffith University, Queensland

ABSTRACT: Since 1985, more than 60 studies have been published in English on the effects of acupuncture on immune function. Modulation of non-specific immunity in response to acupuncture has been demonstrated in a large number of reports. These responses include increases in: the total white blood cell count in peripheral blood, polymorph phagocytosis and the circulating levels of humoral components such as complement, lysozyme, properdin, opsonin, bacteriolysin, serum bactericidin and IFN-gamma. A number of studies have reported increases in the circulating immunoglobulin levels (IgG, IgA, and IgM), as well as, specific antibody levels following acupuncture. Acupuncture has been shown to decrease IgE levels in patients with allergic disease. Acupuncture has also been reported to modulate components of the systemic cellular immune response including: numerical changes to T cell subpopulations, increased T and B cell proliferative responses, increased cytotoxicity, and increased number of natural killer cells.

Recent studies which have examined the effect of acupuncture on cytokines suggest that acupuncture modifies the inflammatory response, particularly in conditions where there is chronic inflammation, and may promote in some instances a shift in the Th1/Th2 balance towards Th1 predominance. Data from both animal models and in human studies often demonstrate decreases in IL-4, IL-6, IL-10, IL-13 and TNF-alpha.

Acupuncture has also been shown to down-regulate neuropeptides (Substance P, CGRP and VIP) and neurotrophins (NGF and BDNF) which contribute to inflammatory response by promoting production of pro-inflammatory and Th2 cytokines and also promoting survival of eosinophils and mast cells. The precise mechanisms of acupuncture in modulating neuropepetides, neurotrophins and cytokines in inflammation remains to be elucidated by future research.

Understanding the effects of acupuncture on immune function is fundamental to clarifying the mechanisms of acupuncture in the treatment of inflammatory, auto-immune, neoplastic and immune-deficient diseases.

Keynote speaker

AACMAC MELBOURNE 2009

The Anti-inflammatory Effects of Acupuncture

John McDonald

School of Medicine, Griffith Health Institute, Griffith Health, Griffith University, Queensland

ABSTRACT: Historical acupuncture literature indicates that acupuncture has been used for milennia to treat numerous inflammatory conditions. Recent research is beginning to elucidate some of the mechanisms underpinning acupuncture's anti-inflammatory effects. These effects include an anti-histamine effect and down-regulation of

pro-inflammatory cytokines (such as TNF-alpha, IL-1 beta, IL-6 and IL-10), and pro-inflammatory neuropeptides (such as Substance P, CGRP and VIP). The involvement of both opioid and non-opioid neurotransmitters has been demonstrated. Of the opioid neuropeptides, mu-receptor ligands such as beta-endorphin, met-enkephalin and endomorphin-1appear to play a prominent role in suppressing inflammation. Neurotrophins (such as NGF, BDNF and NT-1) which can enhance and prolong inflammatory response have also been shown to be down-regulated by acupuncture. Acupuncture has also been found to suppress the expression of COX-1, COX-2 and iNOS during experimentally-induced inflammation. In summary, acupuncture has numerous and profound effects on inflammation which are mediated by a complex neuroendocrinoimmunological network of actions, including anti-histamine and COX-2 inhibiting effects.

Keynote speaker

AACMAC ADELAIDE 2010

The Effects of Acupuncture on Allergic Rhinitis

John McDonald

School of Medicine, Griffith Health Institute, Griffith Health, Griffith University, Queensland

ABSTRACT: A total of 4 systematic reviews have now been published on the clinical efficacy of acupuncture for allergic rhinitis, three of which were published in 2008 or 2009. Reviewers conclusions range between "insufficient evidence" to "safe and effective but more high quality RCTs needed". Evidence of acupuncture efficacy in persistent allergic rhinitis is currently stronger than for intermittent (seasonal) allergic rhinitis.

Evidence for the efficacy of acupuncture in the treatment of allergic rhinitis is underpinned by studies on the mechanisms of acupuncture in modulating immune response in general, more specifically, the anti-inflammatory effects of acupuncture.

An NHMRC-funded study currently in progress at Griffith University on the Gold Coast is examining the effects of acupuncture on mucosal immunity in the upper respiratory tract. In a randomised controlled trial 132 human subjects (aged 18-45 years) are being randomised into 3 groups - routine medication only, routine medication plus sham acupuncture and routine medication plus real acupuncture. The mucosal immune status of subjects is monitored over a 13 week period during which two of the groups receive acupuncture twice weekly for 8 weeks. Clinical measurements include cytokines, pro-inflammatory neuropeptides (SP, CGRP, VIP), neurotrophins (NGF, BDNF), immunoglubulins, rhinitis quality of life questionnaires (RQLQ after Juniper), rhinomanometry, instantaneous total nasal symptom scores (iTNSS) from daily symptom diaries and clinical examination by an allergy specialist. This study has the potential to provide new data on the allergic immune response in humans with persistent allergic rhinitis and to elucidate how acupuncture may modulate it.

Invited speaker

World Federation of Acupuncture-Moxibustion Societies (WFAS) 2010 International Acupuncture Conference in US, San Francisco

Acupuncture and Allergic Rhinitis: Efficacy and Mechanism Research

McDonald, John[1], Cripps, Allan[1], Smith, Peter[1], Smith, Caroline[2], Xue, Charlie (Changli)[3] and Golianu, Brenda[4]

1 Griffith Health, Griffith University
2 CompleMed, University of Western Sydney
3 WHO Collaborating Centre for Traditional Medicine Research, RMIT University, Melbourne
4 Stanford University, California

ABSTRACT: Allergic rhinitis is a common disease in Australia, with an estimated 3.17 million Australians affected. Allergic rhinitis represents a significant burden to the community in quality of life and wellbeing, impaired performance, loss of productivity and health care costs.

A review was untaken firstly of the research literature on the efficacy and effectiveness of acupuncture for allergic rhinitis, then secondly of the research into the mechanisms of acupuncture in allergic rhinitis.

A total of 4 systematic reviews, 13 randomised controlled trials (RCTs) and other non-randomised studies on acupuncture treatment for allergic rhinitis were reviewed. Acupuncture was reported to significantly reduce signs and symptoms of allergic rhinitis in both children and adults. Evidence of acupuncture efficacy for persistent (perennial) allergic rhinitis was reported to be stronger than for intermittent (seasonal) allergic rhinitis.

Multiple physiological pathways appear to mediate the anti-inflammatory effects of acupuncture including the HPA axis, sympathetic pathways, descending inhibitory pathways and possibly parasympathetic cholinergic pathways. Studies have also suggested that acupuncture may down-regulate pro-inflammatory neuropeptides and neurotrophins and may shift the Th1/Th2 balance in T helper cells and hence alter allergic status, however the evidence for these actions is inconclusive.

The proposed study will test whether or not acupuncture can down-regulate pro-inflammatory neuropeptides and neurotrophins or shift the Th1/Th2 balance in adults with allergic rhinitis.

AACMAC PERTH 2011

Problems with Placebo, Sham and Minimal Acupuncture in Research

John McDonald

School of Medicine, Griffith Health Institute, Griffith Health, Griffith University, Queensland

ABSTRACT: The purpose of placebo acupuncture in randomised controlled trials is to measure what part of the demonstrated effectiveness of acupuncture is due to the efficacy of acupuncture, and what part is due to other effects such as positive expectation and belief (placebo effect). Various forms of penetrating and non-penetrating sham protocols have been used in acupuncture trials, but while some sham protocols have been shown to successfully blind subjects, there is evidence to suggest that most sham acupuncture treatments used to date are not physiologically inert. On the contrary, various sham acupuncture methods have been shown to produce significant activations of the brain and brain stem and significant effects on mu opioid receptors. Further, some sham acupuncture protocols have been shown to use different pathways from real acupuncture, and to have different effects on mu receptors from real acupuncture. Frequently the effectiveness of real acupuncture in RCTs is not significantly superior to the effectiveness of sham acupuncture. When sham acupuncture protocols are not inert, it becomes difficult to interpret the results of RCTs as the goal of using placebo controls is to measure the size of the placebo effect within the effects of real acupuncture. When two active treatment protocols (real and sham acupuncture) which have different mechanisms of action are compared in trials, the results of such controlled trials are unable to elucidate either the size of the placebo effect or the efficacy of real acupuncture.

Invited speaker

AACMAC BRISBANE 2012

Future Directions for Acupuncture Research

John L McDonald

School of Medicine, Griffith Health Institute, Griffith Health, Griffith University, Queensland

ABSTRACT: Acupuncture research is at a crossroads. After initially attempting to use the double-blinded placebo controlled model for acupuncture research, eventually the single-blinded placebo controlled model has been widely adopted. However since currently used forms of placebo controls for acupuncture have been problematic, it is possible that a truly inert form of placebo acupuncture may not exist. When placebo protocols are not inert, this introduces negative bias into studies, defeating the original goal of reducing bias. This dilemma is shared by other therapies which involve complex interventions (like psychotherapy), and where skill levels of the practitioner strongly influence clinical outcomes (like surgery). This raises questions about the appropriateness of the current model of Evidence-Based Medicine levels of evidence to assess clinical efficacy for all interventions. Historically the most successful use of the double-blinded placebo controlled trial appears to have been in pharmaceutical trials which have not used complex interventions and in which the skill of the dispenser has not been a significant factor. The Society for Acupuncture Research has proposed a combination of open pragmatic trials along with mechanism studies which they call a "top-down" and 'bottom-up" approach. In addition, open pragmatic studies which compare acupuncture with other therapies could provide useful evidence. Improvements to the systematic review process are proposed, including filters to exclude studies from systematic reviews if the acupuncture treatment or the skill levels of the practitioners who delivered it are deemed to be inadequate. This would require the establishment of standards to assess the quality of acupuncture treatment and practitioner background.

Invited speaker

International Scientific Acupuncture and Meridian Symposium (iSAMS), 2012 – University of Technology Sydney (UTS), Sydney

Acupuncture Research: Current Status and Future Directions

John McDonald

School of Medicine, Griffith Health Institute, Griffith Health, Griffith University, Queensland

ABSTRACT: There are currently over 5,000 randomised controlled trials on the efficacy and effectiveness of acupuncture listed in the Cochrane Central Register of Controlled Trials as well as 39 Cochrane systematic reviews specifically on acupuncture and 223 other reviews listed in the Cochrane Database of Clinical Reviews. Some of these reviews need to be re-evaluated in the light of problems with interpreting placebo/sham - controlled acupuncture studies due to placebo and sham acupuncture protocols being found to be active rather than inert (as was previously assumed). In addition new studies have highlighted measurement errors in previous studies, notably in asthma and tinnitus research.

A significant body of research into mechanisms of acupuncture is helping to explain the effects of acupuncture which have been observed and reported for millennia. The analgesic and anti-inflammatory actions of acupuncture have been the most extensively researched, however a unifying theory of "how acupuncture works" remains elusive.

As advances in medical research into endogenous opioids have helped to elucidate mechanisms of acupuncture analgesia, advances in neuroplasticity research may help to explain how acupuncture can produce long-lasting physiological changes.

Systematic reviews already exclude studies with pOORL methodological quality but may include studies with pOORL acupuncture quality. This negatively skews the conclusions of systematic reviews. Currently there are scales to measure methodological quality (JADAD) and guidelines for reporting (CONSORT and STRICTA) but acupuncture research lacks scales to measure acupuncture quality. The development of an instrument to measure acupuncture quality in studies should be an urgent research priority.

Invited speaker

World Federation of Acupuncture-Moxibustion Societies 8th World Conference on Acupuncture, Sydney, 2013

The effects of acupuncture on mucosal immunity in perennial allergic rhinitis: a randomised, subject-and-assessor-blinded, sham-controlled clinical trial.

McDonald, JL,. Cripps, AW, Smith, PK, Smith, CA, Xue, CL, Golianu, B

Invited speaker

Abstract reprinted in **Australian Journal of Acupuncture and Chinese Medicine Volume 8, Issue 2, 2013**

include conservative treatment, continuous positive airway pressure, oral devices and surgery. In traditional Chinese medicine (TCM), Chinese herbal medicine and acupuncture treatment are the mainstream, but some people are afraid of acupuncture or dislike the bitterness of herbal medicine. Therefore, auricular points treatment might be a third option in TCM. Objective: To study the clinical effect of auricular therapy on OSA, and to find out the constitution of TCM syndrome classification statistics of OSA patients. Methods: The patients were randomly divided into two groups, with 30 cases in the treatment group treated with auricular point sticking and pressing beads and 30 cases in the control group treated with auricular point sticking plaster pressure. The two groups were treated at the same auricular point, once per week. Both groups received one course in total, with two weeks per course. Before treatment two groups filled in sleep questionnaires and TCM constitution assessments; before and after treatment they received monitoring of polysomnography. Results: Comparing the respiratory disturbance index (AHI) before and after treatment, significant differences could be seen in both groups ($P < 0.01$ in treatment group, $P < 0.05$ in control group). Comparing the snore index before and after treatment, significant differences could be seen in both groups ($P < 0.01$). Conclusion: In patients with OSA, constitution types are mainly qi deficiency and phlegm dampness constitution. Auricular therapy can effectively improve AHI and snore index in OSA patients.

Building community trust and protecting public safety: the Australian national registration of Chinese medicine practitioners

By Charlie C Xue (Chinese Medicine Board of Australia)

Background: In 2008 the Council of Australian Governments (COAG) decided to establish a single National Registration and Accreditation Scheme (the National Scheme) for ten health professions. A further four health professions joined the scheme from 1 July 2012, including Chinese medicine. Prior to this, regulation of Chinese medicine practitioners was in place in the state of Victoria only. The National Scheme was established under the *Health Practitioner Regulation National Law Act 2009* (the National Law). There is a National Board for each of the 14 regulated health professions. The Australian Health Practitioner Regulation Agency (AHPRA) is the single agency that supports the National Boards and the National Scheme. Objectives: The role of the Board is to protect the public and set standards and policies that registered Chinese Medicine practitioners must meet. To be registered a practitioner must meet all the Board's registration standards including: criminal history checks; continuing professional development; professional indemnity insurance; recency of practice; English language skills; and grandparenting and general registration eligibility. Other functions of the Board include: handling notifications; assessing overseas trained practitioners who wish to practise in Australia; and approving accreditation standards and accredited courses of study. The presentation will provide a comprehensive update on the regulation of the profession and the progress of the Chinese Medicine Board of Australia. Main results: By June 2013, there were 4070 registered Chinese medicine practitioners in Australia. New South Wales hosts the largest registrant base with 40.52%, followed by Victoria (28.28%) and Queensland (19.29%). Much has been learnt to date. Besides the common challenges, several unique issues such as language, qualification, as well as transitions of legal issues from the former Chinese Medicine Registration Board of Victoria, that the Board has given priority to ensure timely implementation of the practitioner registration process. Conclusion: National registration of the Chinese medicine profession is a landmark development in Australia and the Western developed world.

The effects of acupuncture on mucosal immunity in perennial allergic rhinitis: a randomised, subject-and-assessor-blinded, sham-controlled clinical trial

By John McDonald (Griffith University); Allan Cripps; Peter Smith; Caroline Smith; Charlie Xue; Brenda Golianu

Between 2009 and 2012, 148 adults with perennial allergic rhinitis were recruited and randomised into three groups: real acupuncture, sham acupuncture and no acupuncture. Objectives: The primary objective of the trial was to measure any changes in mucosal immunity after acupuncture, specifically any modulation of pro-inflammatory neuropeptides (SP, VIP & CGRP), any modulation of neurotrophins (NGF & BDNF), and any shift in Th1/Th2 cytokine balance. Secondary outcomes included clinical measures and self-assessment tools such as the Mini rhinoconjunctivitis quality of life questionnaire [MiniRQLQ]. Methods: The real and sham acupuncture groups received acupuncture treatments twice weekly for eight weeks. All groups were assessed by an allergy specialist at baseline and at one and four weeks follow-up. Peripheral venous blood was collected at baseline and four weeks follow-up, and saliva samples were collected at several time-points. Data are presented as mean and standard error of mean. Results: No significant differences were seen in levels of SP, VIP, CGRP, NGF, BDNF or cytokines four weeks after acupuncture treatment. However significant down-regulation in salivary SP was seen 18 to 24 hours after acupuncture (101.09+26.49 pg/ml) compared to pre-treatment (517.95+383.56 pg/ml) ($p = 0.040$). Significant improvements in clinical symptom scores were seen in the real acupuncture group after the first two weeks of acupuncture treatment (Week 1 – 23.27+2.68; Week 3 – 20.33+2.98) ($p = 0.023$) and these improvements persisted

for four weeks after treatment (Week 12 – 17.60+3.37) (p = 0.003). Conclusions: Acupuncture is effective in alleviating symptoms of allergic rhinitis however modulation of neurotrophins, pro-inflammatory neuropeptides and cytokines does not appear to account for this clinical improvement.

The Chinese Medicine Board of Australia's policy on infection prevention and control for acupuncture practice

By Stephen Janz (Chinese Medicine Board of Australia)

Background: The prevention and control of infection is a key professional responsibility of the acupuncture practitioner. In keeping with its primary objective of protecting public health and safety the Chinese Medicine Board of Australia (the Board) has developed Infection prevention and control guidelines for acupuncture practice (the Guidelines). The guidelines are in addition to the NHMRC Australian guidelines for prevention and control of infection in healthcare (the Australian guidelines) which the Board has adopted. The Board's guidelines highlight key features of the Australian guidelines; clarify acupuncture specific areas which are not clearly addressed in the Australian guidelines; and provide emphasis and clarification specific to controlling the risk of infection in acupuncture practice. Aim: The CMBA Guidelines aim to identify specific requirements for the prevention and control of infections in acupuncture practice that are not covered in the Australian Guidelines. The development of the CMBA guidelines take a risk management approach consistent with Australian guidelines and are based upon the best available evidence. Method: The Australian guidelines were examined by the Board to identify areas which required further acupuncture specific guidance. A literature search was then conducted using the State Library of Queensland online search tool and Google using the keywords 'acupuncture' 'infection' 'prevention' 'control'. Limited acupuncture specific infection control literature was identified and one evidence based set of guidelines was found. A further search was conducted for evidence based guidelines for established procedures with a similar procedural risk profile to acupuncture. NHMRC immunisation guidelines and WHO guide to best practice for injections and related procedures were identified. Draft guidelines were developed by the Policies, Standards and Guidelines Advisory Committee (the Policy Committee) based upon the identified evidence based guidelines, revised and approved by the Board for stakeholder consultation. Stakeholder feedback was reviewed prior to finalising the document. Results: Additional guidelines have been developed concerning hand hygiene and the use of alcohol based hand rub; single use of critical items such as acupuncture needles and bamboo cups; appropriate use of gloves; routine skin preparation and post treatment procedures; non-sharp waste disposal; and the prevention and management of sharps injuries. Conclusion: The Guidelines are published on the Chinese Medicine Board of Australia website (www.chinesemedicineboard.com.au) and apply to all Chinese medicine practitioners registered in the division of acupuncture. The Guidelines may be of interest to other health professionals, educators, and regulators concerned with acupuncture practice.

Physiological activities elicited by acupuncture and its sham device in humans and rats

By Kenji Kawakita (Meiji University of Integrative Medicine); Kaoru Okada; Maria Carneiro; Eiji Sumiya; Chie Ogasawara; Yukihito Sugawara; Shigekatsu Aizawa; Syuji Goto

Objective: The purpose of this study was to evaluate the physiological activities of various sham interventions used in recent clinical trials of acupuncture. Such interventions were assumed to be inert. Methods: Neural activities elicited by various acupuncture stimuli, including acupuncture manipulation, press tack needle (PTN), and sham acupuncture stimuli, were recorded electrophysiologically from the afferent fibres in humans and pain-related neurons and/or reward-related nuclei in the rat's central nervous systems. Peripheral nociceptors were classified into C mechano-heat (CMH) units and A mechano-sensitive (AH) units in humans, and central neurons were classified into nociceptive specific (NS) and wide dynamic range (WDR) neurons based on their responsiveness to mechanical and thermal stimuli in rats. The protocols of both experiments were approved by the ethical committee of our university. Results: In humans, all CMH (n = 10) units were activated by real acupuncture, PTN and various sham interventions, but not by sham PTN. In rats, acupuncture manipulation including real PTN activated the NS neurons in the periaqueductal grey matter (PAG, n = 5) and the nucleus accumbens (major nucleus for rewarding system, n = 4). No response was elicited by sham PTN. Conclusion: Various sham interventions used in the published clinical trials could activate the CMH units, presumably C polymodal receptors in human. Only sham PTN could be considered an inert intervention in humans and rats. Real and sham PTN might be useful for future clinical trials of acupuncture.

An innovative approach to individualised acupuncture treatment: preliminary findings of a study on individual variations in endogenous pain controls

By Zhen Zheng (RMIT University); Kelun Wang; Dongyuan Yao; Charlie CL Xue; Genevieve Iversen; Lars Arendt-Nielsen

Introduction: A painful stimulation applied to one site of the body inhibits pain at a distant part. This is called conditioned pain modulation (CPM). The same painful stimulation inhibits pain at or close to the site of stimulation, which is labelled as segmental inhibition (SI). Such controls have been shown to partially explain acupuncture analgesia. Aim: This study aims to

AACMAC MELBOURNE 2014

The effects of acupuncture on mucosal immunity in persistent allergic rhinitis: a randomised, subject-and-assessor-blinded, sham-controlled clinical trial: final results

McDonald, J. L., Cripps, A.W., Smith, P.K., Smith, C.A., Xue, C.C. and Golianu, B.

ABSTRACT: Between 2009 and 2012, 151 adults with persistent allergic rhinitis were recruited and randomised into three groups: real acupuncture, sham acupuncture and no acupuncture.

Objectives: The primary objective of the trial was to measure any changes in mucosal immunity after acupuncture, specifically any modulation of pro-inflammatory neuropeptides (SP, VIP and CGRP), or neurotrophins (NGF and BDNF), and any shift in Th1/Th2 cytokine balance. Secondary outcomes included clinical examinations and symptom and quality of life measures.

Methods: The real and sham acupuncture groups received acupuncture treatments twice weekly for 8 weeks. All groups were assessed by an allergy specialist at baseline and at one and four weeks follow-up. Blood and saliva samples were also collected.

Results: Significant down-regulation in SP was seen 18 to 24 hours after acupuncture in Week 1 (by 83.2%; $p = 0.039$) and Week 3 (by 85.5%; $p = 0.041$) compared to baseline. Total IgE (17.9% decrease; $p = 0.016$) and specific IgE for house dust mite (24.0% decrease; $p = 0.035$) were significantly reduced at four-weeks follow-up compared to baseline. Significant improvements in clinical symptoms were seen after the first two weeks of acupuncture treatment and these improvements persisted at four weeks follow-up. Relief medication use was also reduced at four-weeks follow-up ($p = 0.034$). No significant differences were seen in nasal airway patency, or levels of SP, VIP, CGRP, NGF, BDNF or cytokines at four-weeks follow-up.

Conclusions: Acupuncture is safe and effective in improving symptoms and quality of life and reducing use of relief medications in adults with persistent allergic rhinitis. However these clinical improvements did not correlate with any observed changes in nasal airway patency, neurotrophins or cytokines. Further research is needed to clarify the potential roles of SP and VIP in mediating the effects of acupuncture in allergic rhinitis.

Invited speaker

Best Research/Scientific Paper award

APPENDIX C

iTNSS (instantaneous total nasal symptom score). Please circle

A. Runny Nose

0	1	2	3
Not at all	Mild	Moderate	Severe

B. Itchy Nose

0	1	2	3
Not at all	Mild	Moderate	Severe

C. Stuffy Nose

0	1	2	3
Not at all	Mild	Moderate	Severe

D. Sneezing

0	1	2	3
Not at all	Mild	Moderate	Severe

APPENDIX D

Study Administrative Centre:
Allergy Clinic
Telephone +61 (0) 7 5591 5744 indicating that you are enquiring about the Acupuncture Study
Email: confirmations@aapt.net.au
5/123 Nerang Street
Southport, QLD, 4215 Australia

OR

Acupuncture Clinic
Telephone +61 (0) 7 5571 1166
G/4 Railway St
Southport, QLD, 4215 Australia

STUDY TITLE
Acupuncture and mucosal immunity in the upper respiratory tract

MINI RHINOCONJUNCTIVITIS QUALITY OF LIFE QUESTIONNAIRE (Mini RQLQ)

SELF-ADMINISTERED
ENGLISH FOR AUSTRALIA VERSION

TO BE COMPLETED AT WEEK 0

MINI RHINOCONJUCTIVITIS

QUALITY OF LIFE QUESTIONNAIRE PATIENT NO._____

(English for Australia version) **DATE:_____**
Self-administered

Please complete **all** questions by circling the number that best describes how affected you have been during **the last week as a result of your nose/eye symptoms**

	Not Affected	Hardly affected at all	Slightly affected	Moderately affected	Quite a bit affected	Very affected	Extremely affected
ACTIVITIES							
1. REGULAR ACTIVITIES AT HOME AND AT WORK (your occupation or tasks that you have to do regularly around your home and/or garden)	**0**	**1**	**2**	**3**	**4**	**5**	**6**
2. RECREATIONAL ACTIVITIES (indOORL and outdOORL activities with friends and family, sports, social activities, hobbies)	**0**	**1**	**2**	**3**	**4**	**5**	**6**
3. SLEEP (difficulties getting a good night's sleep and/or getting to sleep at night)	**0**	**1**	**2**	**3**	**4**	**5**	**6**
PRACTICAL PROBLEMS							
4. NEED TO RUB NOSE/ EYES	**0**	**1**	**2**	**3**	**4**	**5**	**6**
5. NEED TO BLOW NOSE REPEATEDLY	**0**	**1**	**2**	**3**	**4**	**5**	**6**

MINI RHINOCONJUCTIVITIS
QUALITY OF LIFE QUESTIONNAIRE PATIENT NO._____

(English for Australia version) DATE:_____
Self-administered

Please complete **all** questions by circling the number that best describes how affected you have been during **the last week as a result of your nose/eye symptoms.**

Not Affected	Hardly affected at all	Slightly affected	Moderately affected	Quite a bit affected	Very affected	Extremely affected

NASAL SYMPTOMS

6.	**SNEEZING**	**0**	**1**	**2**	**3**	**4**	**5**	**6**
7.	**STUFFY/BLOCKED NOSE**	**0**	**1**	**2**	**3**	**4**	**5**	**6**
8.	**RUNNY NOSE**	**0**	**1**	**2**	**3**	**4**	**5**	**6**

EYE SYMPTOMS

9.	**ITCHY EYES**	**0**	**1**	**2**	**3**	**4**	**5**	**6**
10.	**SORE EYES**	**0**	**1**	**2**	**3**	**4**	**5**	**6**
11.	**WATERY EYES**	**0**	**1**	**2**	**3**	**4**	**5**	**6**

OTHER SYMPTOMS

12.	**TIREDNESS AND/OR FATIGUE**	**0**	**1**	**2**	**3**	**4**	**5**	**6**
13.	**THIRST**	**0**	**1**	**2**	**3**	**4**	**5**	**6**
14.	**FEELING IRRITABLE**	**0**	**1**	**2**	**3**	**4**	**5**	**6**

APPENDIX E

MONTH 1 (Month of recording_____)

Patient Identification Number_____Date of birth _____

DAILY SYMPTOM AND MEDICATION RECORD SHEET

Please tick the appropriate box or boxes each day.

DATE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Nasal itch																															
Eye itch																															
Sneezing																															
Runny nose																															
Post nasal drip																															
Unrefreshed sleep																															
Sinus pain																															
Medications (please provide details)																															
Other comments																															

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