A Clinico-Pathological Investigation of Rosacea with Particular Regard to Systemic Diseases

Dr. Mustafa Hassan Marai

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“I can confirm that the work submitted is my own and that appropriate credit has been given where reference has been made to the work of others”

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Dr. Mustafa Hassan Marai

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Abstract

Background: Rosacea is a common skin disorder that predominantly affects fair skinned people, particularly of Celtic origin. It usually presents with erythema, telangiectasia, and papulo-pustular lesions on the face and chest and usually triggered by sun exposure. The finding of a positive anti-nuclear antibody (ANA) has been reported in rosacea, as a consequence, those rosacea patients may be mislabeled as lupus erythematosus, often with important consequences in terms of treatment, and presumed prognosis. There is a limited literature examining rosacea and its associations with the positivity of ANA and connective tissue diseases (CTD).

Objectives: This study investigated the relationship of different sub-types of rosacea with positivity of ANA test, musculo-skeletal systemic symptoms including myalgia, arthralgia and Raynaud's phenomenon and CTD particularly lupus.

Method: This was principally an observational study, I investigated a large group of patients (169 patients) with different subtypes of rosacea, identified from the dermatology and rheumatology departments in Doncaster Hospitals (93 patients) and Leeds Hospitals (76 patients). All patients had ANA blood screening test and all required data about their rosacea, associated systemic symptoms and previous history of CTD were recorded in special proformas after patients read information leaflet sheet and signed participation consent form.

Results: The results showed no significant increase in the ANA positivity test (overall 13%), however, in patients without a history of CTD, the level of ANA positivity of both centres combined was (5.3%) which is similar to that reported in the general population; (One-sample Binomial test compared to null hypothesis proportion [5%] p=0.500). Around 15 - 20% of patients had one or more systemic symptoms. Arthralgia and myalgia had the same percentage as reported by the control group and in normal populations; however, Raynaud's phenomenon was slightly greater than reported in the

control group and the general populations. There could be an inverse relationship between Raynaud's treatment with vasodilators and rosacea flushing symptom.

Conclusion: This study confirmed that there is no evidence that any particular clinical sub-type of rosacea is associated with increased positivity of ANA or has specific relationship with CTD. The study also did not find any specific relationship between rosacea and systemic symptoms.

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Table (1) - Abbreviations

No	Abbreviated ward	Explanation
1	ANA	Anti-nuclear antibody
2	CTD	Connective tissue disease
3	NRS	National Rosacea Society
4	UV	Ultraviolet light
5	UVA	Ultraviolet A
6	UVB	Ultraviolet B
7	DIF	Direct Immunofluorescence
8	IIF	Indirect Immunofluorescence
9	TLR	Toll-like receptor
10	ER	Endoplasmic reticulum
11	VEGF	Vascular endothelial growth factor
12	DF	Demodex folliculorum
13	HP	Helicobacter pylori
14	IL	Interleukin
15	TNF-α	Tumour necrosis factor alpha
16	ETR	Erythematotelangiectatic rosacea
17	PPR	Papulopustular rosacea
18	PR	Phymatous rosacea
19	OR	Ocular rosacea
20	GR	Granulomatous rosacea
21	MMP	Matrix metalloproteinase
22	ROS	Reactive oxygen species
23	NO	Nitric oxides
24	AMP	Anti-microbial peptide
25	EGFR	Epidermal growth factor receptor
26	LE	Lupus erythematosus
27	CLE	Cutaneous lupus erythematosus
28	ACLE	Acute cutaneous lupus erythematosus
29	SCLE	Subacute cutaneous lupus erythematosus
30	CCLE	Chronic cutaneous lupus erythematosus
31	SLE	Systemic lupus erythematosus
32	DLE	Discoid lupus erythematosus
33	ENA	Extractable nuclear antigen

Chapter One - Introduction

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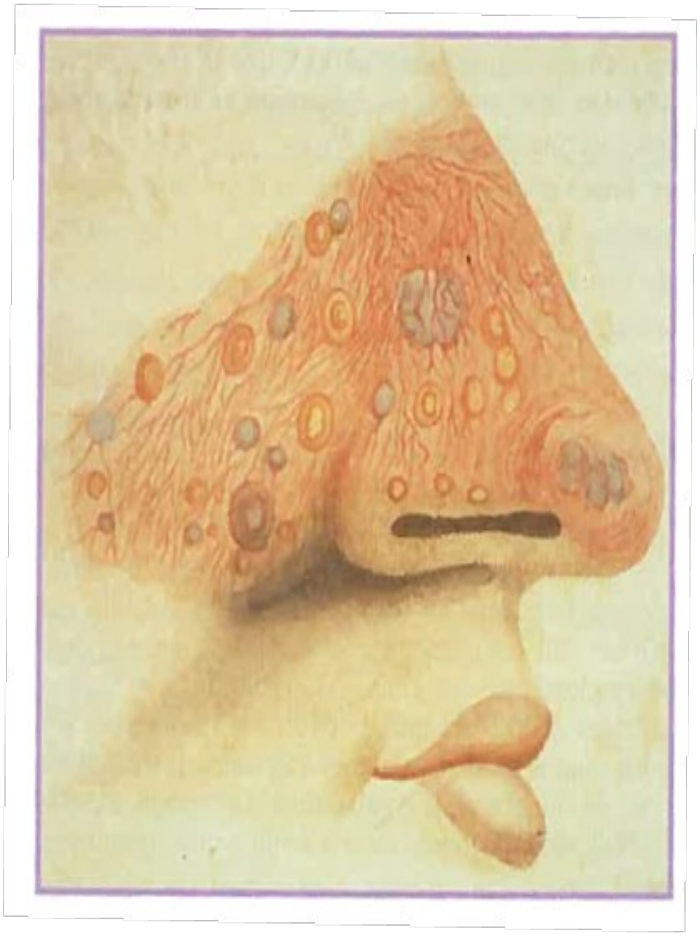


1. Background

This thesis has its origins in a clinic scenario well recognised by those Dermatologists working in the field of connective tissue disease.

A female patient of 38 was seen with a prior diagnosis of lupus erythematosus. Her current complaint was that her facial skin rash, on which the diagnosis of lupus was based, had failed to respond to a number of treatments commonly used for cutaneous lupus including antimalarials, dapsone, and oral gold. Her skin rash was papular, but never pustular and intermittently itchy. It was aggravated by exposure to ultraviolet light (UV) and she had mild arthralgia affecting a number of small joints. She had an intermittently present ANA at low titre 1:80. A skin biopsy was reported as being compatible with cutaneous lupus. At the time the patient was examined, she had a background erythema of her face, with a widespread papular eruption affecting the central face. There were no pustules, and the eruption was relatively monomorphic. She had aching finger joints but no joint swelling. Her nail fold capillaries were normal, and no other abnormal physical signs were found. Her ANA was positive at a titre of 1:80. A repeat skin biopsy showed a follicular eruption, with a predominantly lymphocytic histology, no basal layer degeneration nor basement membrane thickening. A specialist dermato-pathologist reported the histology as being unequivocally rosacea, and certainly not lupus. This raised the question of whether the patient had both lupus and roscaea. A review of the original pathology showed changes very similar to the recent biopsy, suggesting that the diagnosis of the facial rash had been rosacea all along. Treatment with oral tetracycline produced complete resolution of the skin eruption. The arthralgia responded to anti-inflammatory agents, and the ANA continued to be intermittently present. The patient did not have lupus, but the question of the interaction between these two conditions was raised, and merited further investigation.

The diagnosis of rosacea is often an easily made clinical one, but other differential diagnosis conditions may need biopsy (see later), and some clinico-pathological correlation to ensure that the right choices of diagnosis and therapy are made. It is not surprising that the combination of symptoms and signs seen in the patient described above may lead to a diagnosis of lupus of the predominantly cutaneous type, and may often be correct. However, as this case indicates, even a skin biopsy may be misinterpreted if the reporting histo-pathologist is inappropriately 'guided' by the clinician. The microscopic changes in both rosacea and lupus are predominantly dermal and lymphocytic, so confusion is easily explained.

There is a small literature concerning the frequency of the presence of a positive ANA in patients with rosacea, and in some studies (see later), the frequency is higher than in a 'normal' population, and may occur in association with other immunological abnormalities. Rosacea is also frequently aggravated or precipitated by physical stimuli, including UV exposure. There is sufficient commonality therefore for the separation of these two conditions; rosacea and cutaneous lupus to be difficult, even for those experienced in the dermatological aspects of CTD. Since many such patients are often under the care of non-dermatologists, it would be surprising if there was not occasional mis-diagnosis, or at least over-interpretation of the meaning of combinations of symptoms and signs. In any specialist practice, it is difficult to be certain, until appropriate research is carried out, whether an observation made is real and generalisable, or is specific to the area of work of the observer, or indeed is not 'real' at all, but a function of the heightened suspicions of the observer. The observation that the diagnosis of rosacea and lupus was complicated by the apparent increase in frequency of ANA positivity, as well as the occurrence of relatively non-specific joint and muscle symptoms in those with rosacea, led to the generation of this piece of work. Although

the increase in ANA positivity in rosacea has been described, the studies are few and the numbers of patients small, and so was this a real and generalisable finding? If so, was it associated with extra-cutaneous symptoms, and what was the relevance of these findings if they were genuine? Did these features indicate a sub-clinical CTD, or did they indicate that rosacea and CTD were related in some way, possibly through the impact of treatment? It is well known that topical or oral steroid use can produce a rosacea like cutaneous eruption, and that certain tetracycline antibiotics can produce a lupus-like illness.

With all of this in mind, the research described in this thesis was designed and carried out as an extended piece of data collection and clinico-pathological correlation, with patient numbers sufficient to answer the key questions. The involvement of two quite different out-patient clinics, one a general clinic in Doncaster District General Hospital, the other in a specialist unit in a large Leeds Teaching Hospital, allowed comparisons between two cohorts with similar demographics, but different disease backgrounds, whilst also producing valuable combined data. A control group of non-rosacea patients allowed standardisation against published norms, and provided useful control data where none existed previously. Data collection was prolonged, since finding patients who fitted the inclusion criteria was less easy than anticipated, but the planned numbers were achieved, and the questions addressed.

2. History of Rosacea (1)

“ … I never think of your face but I think upon hell fire … " **Shakespeare (Henry IV)**

“ … Rosacea, a nice name for an unpleasant complaint ... " **John Banville (The Sea)**

Figure 1 - Illustrated figure of rosacea in nineteenth century (1)

Robert Willan (1757 – 1812) an English Dermatologist, originally described rosacea in exacting detail. He practiced at the Carey Street dispensary in London. He introduced the term acne rosacea as a type of acne that initially affects those in middle age and which presents with facial papules and pustules on a background of red skin. He was the first to mention the inflammation of the eyes in patients with acne rosacea. His description of rosacea was published by his student Bateman one year after Willan’s death, in his book on the classification of diseases of the skin in 1813. In 1800, other dermatologists described rosacea as a skin condition with a marked redness of the face.

It was thought to result from continual heat exposure and at that time was called lichen agrius. Jean-Louis Alibert (1768 - 1837) and Alphonse Devergie (1798 - 1879) both French dermatologists, working in the Hospital Saint-Louis in Paris, classified rosacea in the group of dermatoses with sebaceous gland pathologies within which was also included acne vulgaris. Erasmus Wilson (1809 - 1879) an English dermatologist suggested that there was a common pathogenesis of acne rosacea and acne vulgaris. Paul Gerson Unna (1850 - 1929) a German physician in Hamburg, one of the famous dermatologists of the early decades of the 20th century, supported the hypothesis of sebaceous gland dysfunction in rosacea. Radcliff-Croker (1845 - 1909) an English dermatologist, considered the term acne rosacea was inappropriate. He suggested that the erythema of acne rosacea was not a result of inflammation of the pilosebaceous glands, but rather that it was the result of abnormal dilation of facial blood vessels, leading to leakage of fluid into perivascular dermal tissue and resulting in a reactive inflammatory papulopustular skin eruption. He and other English dermatologists recommended dropping the ward acne and simply naming the condition rosacea, a disorder completely different from acne vulgaris. They also postulated that chronic facial blood vessel congestion could lead to hypertrophic tissue changes and in this way to the end stage of rosacea. This theory proposed that rosacea started with a red stage (erythematotelangiectatic) and was followed by an inflammatory stage (papulopustular) and ended in hypertrophic tissue changes, the phymatous stage. This hypothesis remains broadly accepted today, although it is clear that not all patients with papulopustular disease have necessarily started with erythematotelangiectatic disease.

3. Definition

Rosacea is a common chronic skin disorder in which follicular inflammation and vascular instability occur, together or alone, and that may be followed by phymatous change. It is poorly understood despite its frequency, often misunderstood and lacks satisfactory definition in a number of circumstances. It usually affects facial skin and less commonly may affect the neck and chest; it may also occur at non-facial sites. It is characterized in its early phases by erythema (flushing and redness) on the central face and across the cheeks, nose, or forehead.

As rosacea progresses, other symptoms can develop such as semi-permanent or permanent erythema, telangiectasia, red domed papules and pustules, red gritty eyes, and burning and stinging sensations. In some advanced cases, rosacea can be complicated by chronic skin lymphoedema, thickening of the affected skin and a red lobulated nose (rhinophyma). Not all patients have all elements of the condition: some have only the vascular elements, whilst others have a predominantly papular or pustular version without much vascular hyper-reactivity. Occasionally, the condition may occur at sites away from the face, when the diagnosis may be less obvious, but the morphology of the skin lesions remains consistent. The disorder can be confused and co-exist with acne vulgaris or seborrheoic eczema.

Rosacea is predominantly a clinical diagnosis and whilst histology is helpful to assist in diagnosis, and to exclude other conditions, there is no benchmark laboratory test to confirm diagnosis. In 2002 an expert committee assembled by the National Rosacea Society (NRS) in the USA set up diagnostic clinical criteria for rosacea. The expert panel also recognised 4 subtypes of rosacea (see later) (2).

4. Epidemiology

Rosacea is a common skin disease, however the epidemiological data remain fragmentary and the methodological quality of many studies is debatable. The prevalence statistics published in Europe and the United States are highly variable from one study to another, depending on the method used and populations studied. Published results range from 1% to 20% of the adult population (3). Rosacea affects both sexes, but is almost three times more common in women aged 30 to 50 years, although the development of the complication of rhinophyma is more common in men. It has a peak age of onset between 30 and 60 years, but it can occur rarely in children (4). Rosacea predominantly affects white-skinned people of mostly north-western European descent, particularly those of Celtic origin; however it can occur in any race (5).

In one hospital-based study, rosacea made up 2% of all patients who consulted dermatologists (6). A Swedish epidemiology study, in 1989**,** investigating 809 office workers (454 women and 355 men), showed that 81 persons were diagnosed with rosacea, giving the prevalence of 10% (women 14% and men 5%) (7). In another epidemiological study from Ireland, the country which is thought to have a high incidence of rosacea, the prevalence of rosacea was 13.9% (8). The prevalence rate in these two studies was high and this is probably reflects the patients skin type (Celtic skin origin). A further study from Estonia, in 2010 reviewed the prevalence of rosacea in 348 participants selected randomly from a working population of 30 years of age or more. They used the NRS diagnostic criteria. The results showed that 78 candidates (22%) had one or more primary features of rosacea (9). The prevalence was high, this is could be result from patients selections, where age of patients involved were above 30 years, as well as the flushing was not considered a prerequisite for diagnosis of rosacea subtype 1. Furthermore, the numbers of participants were small.

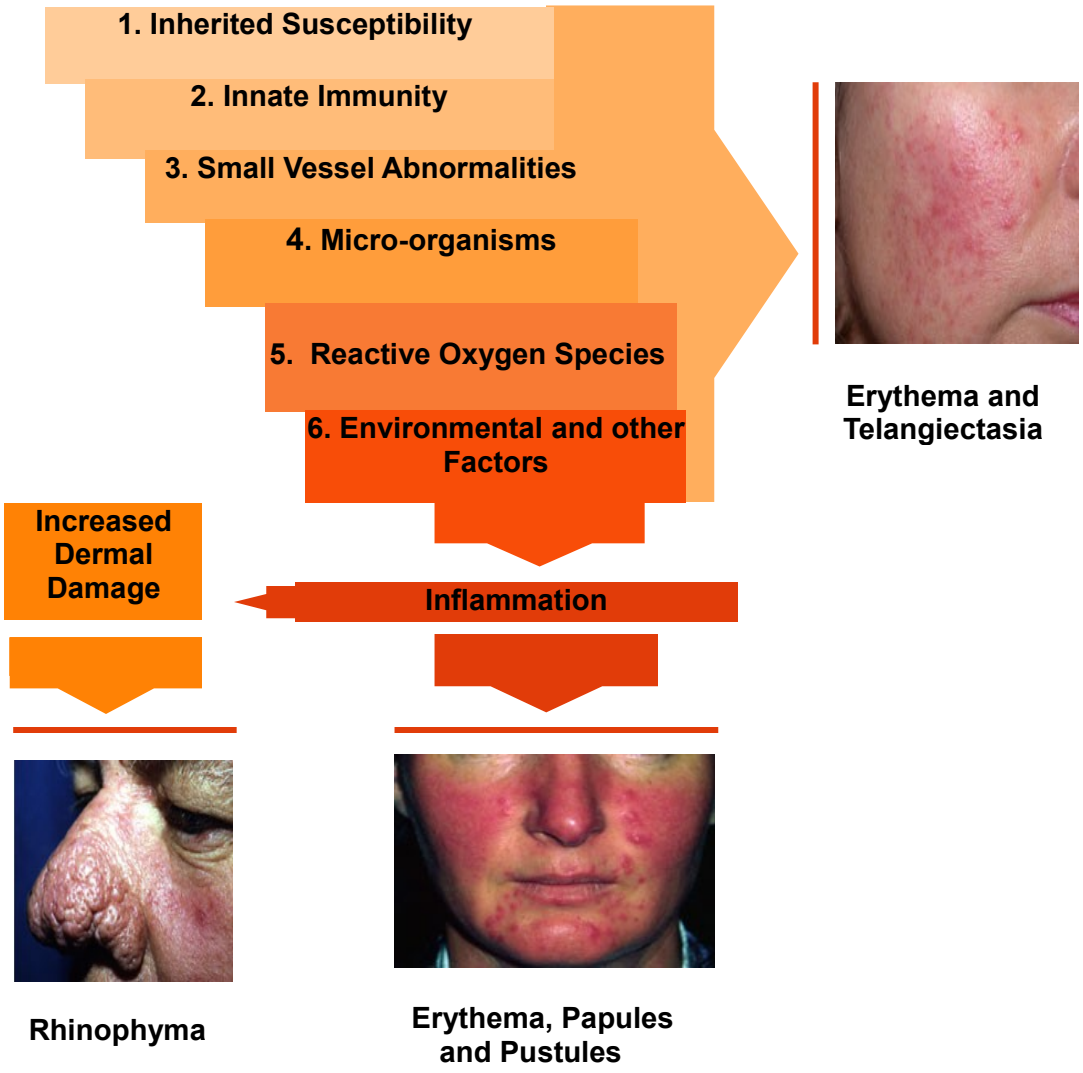
A retrospective study from Tunisia, in 2010 included 244 patients. The prevalence of rosacea was 0.2%, with a sex ratio of male (69 patients) to female (175 patients) of 0.4. The mean age of patients was 49 years (10). There is clearly a very large difference between these prevalence figures and those of the other studies. The explanation is unclear, but may be related to racial factors. In a recent study of the epidemiology of rosacea in UK in 2012**,** where the data was collected from UK General Practice Research Database identified patients with an incidental diagnosis of rosacea between 1995 and 2009 and matched them (1:1) to rosacea free control patients. The total identified number of rosacea patients was 60,042 with 60,042 controls. The overall incidence rate was 1.65 per 1000 person - years and 61.5% were women (11). These figures are much closer to those seen in the earlier study from Tunisia, and feel more realistic than higher figures reported from other studies.

There are many discrepancies in the results of the epidemiological studies performed over the last few decades. These may have resulted from the differences in methodology including patients selections and diagnostic criteria used as well as populations, skin type, geographical areas with the effects of environmental factors, cultural and social perceptions of the disease. Also the epidemiology may be affected by the different access to the different health services among the different countries, where some countries only have private health sector while other countries have free governmental health services and others have both. This may affect patient participations and recruitment, research data collection, as well as funding and performing epidemiology research studies.

5. Aetiopathogenesis

The precise aetiology of rosacea is still unknown, and a multi-factorial aetiology is likely. Over the years, many suspected but unconfirmed factors have been reported. These include genetic predisposition, innate immunity, vascular abnormalities, micro-organism infestation such as demodex folliculorum, helicobacter pylori, reactive oxygen species, environmental factors and hormonal changes. None explain the disease entirely.

Figure 2 - Hypothetical Sequences of Rosacea Development: (2)



5.1 - Genetic:

Although there are many triggering factors which may be implicated as a cause of rosacea, these triggers are also experienced by healthy persons who never go on to develop the symptoms or signs of rosacea. Therefore, rosaceous individuals may have an inherent sensitivity to these triggering factors, and this predisposition may be genetic. The evidence for a genetic predisposition in rosacea is not proved and no specific gene defect has been discovered.

In a survey from the NRS of 2052 rosacea patients, about 40% of respondents gave a strong family history of rosacea, 27% confirmed that one or both of their parents suffered from rosacea, 18% had a brother or sister having a history of rosacea, 13% had their grandparent suffering from rosacea, 16% had their aunt or uncle affected with rosacea, 11% had rosacea already diagnosed in their sons or daughters. Sixty percent of the respondents had fair skin with 33% having at least one parent of Irish ancestry, 27% had one of their parents of Scandinavian origin and 26% had a parent of English descent (12). Another survey from the NRS of 600 rosacea participants showed that 52% had a positive family history of rosacea mainly affecting fathers (35%) followed by mothers (30%) and sisters (24%) (some patients had more than one member of the family affected). There were 42% originally from Irish, German or English nationality (13).

These surveys show that rosacea is a skin disease that runs in families and that is also more common in people of certain nationalities. It is likely that there is a genetic element in these areas of enhanced risk.

5.2 - Innate Immunity:

The role of innate immunity in the pathogenesis of rosacea has been studied widely over the last few years. The recognition system of the Innate Immunity includes TLR (Toll-like receptors) responds to environmental stimuli such as UV, microbes, physical and chemical trauma and leads to a controlled increase in cytokines and anti-microbial molecules in the skin. One of these anti-microbial molecules (anti-microbial peptide AMP) is a peptide called cathelicidin, which is known to have both vasoactive and pro-inflammatory actions (14). Cathelicidin is expressed in keratinocytes, leukocytes such as neutrophils, monocytes and natural killer (NK) cells and in epithelial cells of the skin. In the skin, cathelicidin is processed by serine proteases of the kallikrein family particularly kallikrein 5 (stratum corneum tryptic enzyme) and kallikrein 7 (stratum corneum chymotryptic enzyme) (15). The main resulting peptide is LL-37; however, LL-37 can be processed further to smaller peptide fragments. These smaller peptide fragments exert immune functions but differ in their antimicrobial and immune activating capacities (16).

A number of studies have investigated the role of the innate immune system, and cathelicidin in particular in rosacea. In one study, it was shown that cathelicidin is significantly increased in the lesional skin in rosacea compared to the skin of non-affected individuals. Also injection of these peptide fragments, found in the skin of rosacea patients, into the skin of mice leads to a rosacea-like disease. In contrast, the isolated increase of protease activity in cathelicidin knock-out mice does not cause dermal inflammation (17).

These cathelicidin peptides promote and regulate leukocyte chemotaxis (18), angiogenesis (19), and expression of extracellular matrix components (20), so would be ideally placed to have a role in rosacea.

The mechanisms underlying the increased cathelicidin production and the enhanced protease activity in skin of the rosacea patients are not well known. Both seem to be regulated by different signalling pathways with retinoid, vitamin D- and cytokine activated cascades playing important roles (21). Cathelicidin LL-37 expression in human keratinocytes is regulated by the vitamin D pathway and this could explain why rosacea occurs mainly in the sun exposed areas as exposure to UV light triggers activation of vitamin D in keratinocytes and subsequent cathelicidin expression (22). Recently, a second vitamin D independent pathway triggering the induction of cathelicidin synthesis in keratinocytes was identified. In keratinocytes, cathelicidin expression increases upon several external stimuli such as infection, injuries, UV irradiation, and permeability barrier disruption which also trigger endoplasmic reticulum (ER) stress. The ER stress increases cathelicidin expression via nuclear factor KB-carbohydrate responsive element binding protein α activation independent of vitamin D receptor (VDR) activation demonstrating a novel role for ER stress in stimulating innate immunity (23). This again could explain why rosacea patients often report on nonspecific triggers (e.g. heat) which would mediate their pro-inflammatory activities through ER stress and cathelicidin induction.

The keratinocytes express elevated level of TLR2 resulted from chitin released from demodex mites' colonization in rosacea skin. This leads to further higher expression of kallikrein proteases and higher protease activity. Thus, decreasing the load of demodex mites on the skin or blocking the TLR2 by retinoids could reduce the protease activity and consequently reduce the rosacea inflammation. Also oral tetracycline and topical azelaic acid inhibit protease activity and exert their anti-inflammatory effect through this mechanism (24).

Figure 3 - Role of cathelicidin in the pathogenesis of rosacea: (24)

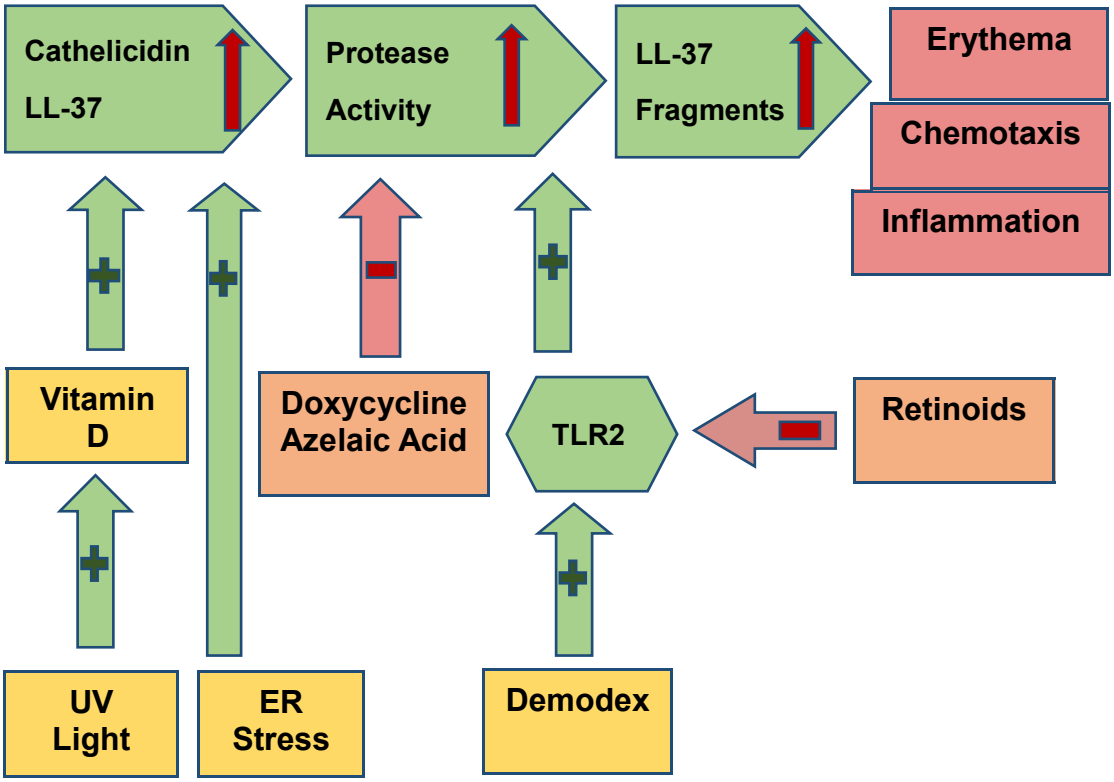


Figure 3 shows the role of cathelicidin in the pathogenesis of rosacea. UV light increases the synthesis of vitamin D which induces cathelicidin expression in keratinocytes. ER stress is an alternative inducer of cathelicidin production. Demodex mite colonization increases the protease activity through the activation of TLR2 receptor which leads to increased cleavage of cathelicidin LL-37 and further fragments. The increased level of the vasoactive and inflammatory host-defense peptide LL37 and its fragments result in rosacea changes including erythema, telangiectasia and inflammation. Oral doxycycline, topical azelaic acid and oral retinoids have their inhibitory effect on the expression and production of cathelicidin through different mechanisms.

5.3 - Vascular Abnormalities:

Many believe that rosacea may be a predominantly vascular disorder because of its association with flushing, redness and visible blood vessels, and certainly, the majority of patients appear to have a vascular element to their disease. The association of rosacea with migraine seems to support this hypothesis (25) as well as the fact that factors that trigger flushing such as emotional stress, spicy food, alcohol and hot beverages may all worsen rosacea. The flushing response or transient erythema is mediated by release of vasoactive substances including Serotonin, Bradykinin, Prostaglandins, Substance P, Opioid peptides and Gastrin (26); however, there is still a lack of convincing supporting experimental evidence of the role of these soluble substances.

There is an increase in the expression of vascular endothelial growth factor (VEGF) which is induced by ultraviolet irradiation and produced in the keratinocytes in rosacea. This may explain the photo distribution and the reported photo aggravation of rosacea. VEGF stimulates the proliferation of vascular endothelial cells and increases angiogenic processes. This may be implicated in the production of erythema in rosacea. It also increases permeability of the blood vessels as well as the expression of the lymphatic endothelial marker D2-40 (27).

In addition, as previously discussed, the cathelicidin-derived peptide LL-37 has been shown to be increased in papulopustular rosacea. It exhibits angiogenetic activity and can induce alteration in endothelial cells via multiple signalling pathways. These include promotion of angiogenesis with neovascularization mediated by interaction with endothelial cell receptors, receptor transactivation with downstream signalling in epithelium and receptor mediated induction of VEGF in keratinocytes (28)

There have been studies confirming the vascular changes in rosacea. In one such study, it was demonstrated that the blood flow in rosacea lesions was 3-4 times that of controls measured by Laser Doppler Flowometry (29). A further study, investigating the association of neurovascular and neuroimmune changes in different clinical presentations of rosacea using quantitative real-time polymerase chain reaction (PCR) and immunohistochemistry, supports the major presence of vasodilation of blood vessels and lymphatics. It also demonstrates the up-regulation of genes involved in vasodilatation, and supports the observation that blood vessels in rosacea retain their ability to respond to vasoactive stimuli (30). Rosina et al, investigated the changes in the cutaneous vasculature on the facial cheek skin of 30 patients with erythematotelangiectatic rosacea by videocapillaryscopy and these changes were correlated with clinical observation by both clinicians and affected patients. The larger vessel diameter, more prominent telangiectasias, neoangiogenesis, and larger capillary nets were noted to be more frequent in those patients compared to healthy controls (31).

So, all the above studies showed that either there is an increase in the blood flow, dilatation of blood vessels or formation of new blood vessels (telangiectasia) in rosacea skin which, all result in the erythema and the redness features of rosacea.

Table 2 - Pathophysiologic Vascular Changes in Rosacea: (32)

Intermittent Flare Episodes with Diffuse Erythema	Intermittent Flare Episodes + Early Persistent Diffuse	Intermittent Flare Episodes + Advanced Persistent Erythema
- Altered blood flow.	- Increase in VEGF stimulation of vasculature.	- Permanent dilatation of the superficial vessels.
- Neurovascular / neuroimmune deregulation.	- Repeated endothelial nitric oxide (NO) stimulation leads to vasodilatation.	- More fully developed fixed structure changes.
- Augment innate immune response (cathelicidin) leads to inflammation.	- Activated cathelicidin cascade leads to more inflammation and angiogenesis.	- Facial cheek skin with sub-papillary plexus dilatation, with thickened walls, enlarge telangiectasias and increase neoangiogenesis.
- Increased blood flow in the affected skin more than none affected skin.	- Vascular / dermal matrix degeneration leads to more angiogenesis	

Table 2 demonstrates the progression of vascular changes and physical alterations of cutaneous vasculature that develop over time in rosacea and their correlation with visible manifestations.

5.4 - Micro-organisms:

- **Demodex Folliculorum (DF):**

Demodex are considered to be commensal organisms in human skin and their numbers increase with the host age. The tendency of rosacea to develop after age of 30 years is paralleled by an increase in demodex mites in facial skin. Many authors believe that demodex folliculorum (DF) has some role in the rosacea development and many studies support this argument.

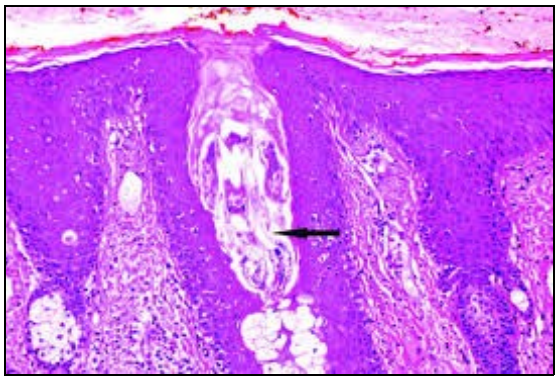


Figure 4 - Demodex mites in skin biopsy

A comparative study, compared standardized skin surface biopsy (1cm²) performed in 49 rosacea patients. The density of DF was significantly higher in patients with rosacea (mean = 10.8/cm²) than controls (mean = 0.7/cm², $p < 0.001$). This density was most statistically significantly higher than in controls in the PPR patients (mean = 12.8/cm², $p < 0.001$). This study demonstrated a high density of DF in rosacea particularly in PPR (33). In a case control study, the prevalence of DF mites was investigated in facial biopsies of 75 patients with rosacea as the case group, and compared with 75 patients with discoid lupus erythematosus and 75 patients with actinic lichen planus as control groups. The prevalence of DF mites in patients with rosacea (38.6%) was significantly higher than the patients with discoid lupus

erythematosus (21.3%) and actinic lichen planus patients (10.6%) ($p < 0.001$). This study suggests that DF mites were increased in rosacea skin compared to other skin dermatoses which indicate that it might play a role in pathogenesis of rosacea (34). In another study investigated 38 rosacea patients and 38 age and sex matched healthy control using skin surface biopsy technique from three facial sites. The mean DF mite count in the rosacea group (6,684) was significantly higher than that in controls (2,868, $p < 0.05$). The cheek was the most heavily infested facial region. In conclusion large numbers of DF may be found in rosacea and may have an important role in the pathogenesis of the disease (35). In a multicentre, cross sectional prospective study, 50 rosacea patients were compared with 48 age and sex matched healthy volunteers. The quantity of DF was measured by PCR. Inflammatory and immune markers were also assessed. The results showed that Demodex was detected more frequently in rosacea patients (5.7 times) than controls (96% vs. 74%, $p < 0.01$). Skin sample analysis showed a higher expression of genes encoding pro-inflammatory cytokines (IL-8, IL-1b, TNF-α) in rosacea, especially PPR. Also over expression of LL-37 and VEGF were observed, indicating broad immune system activation in patients with rosacea (36).

All of the above studies confirmed that the density number of DF in rosacea skin increased compared to healthy controls or other skin dermatosis, however the number of patients studied were small but it is an interesting finding.

On the other hand many other authors argue that although the DF frequently isolated in the rosacea skin and an immunological response against demodex may be detected in those patients, the prevalence of this microorganism in healthy adults reaches up to 100%, and because of this fact, its association with rosacea does not imply an etiopathogenic relationship (37). Also clearing of rosacea physical signs after oral tetracycline did not affect the resident DF population as well as topical application of sulfur ointment improves rosacea without affecting the mite density

number (38). Furthermore, it is impossible to establish the pathogenesis of DF by producing experimental infestation according to the following Koch postulates (39):

1. Demodex is a parasite of healthy skin.
2. Pathogenicity depends on the immunological background of the patients.
3. It is an obligate parasite, and cannot be grown in vitro.

So, in conclusion although it has been found that DF is substantially more numerous in rosacea patients, it is still unclear whether this is a cause or a result of rosacea. It is not clear whether rosacea provides a suitable environment for multiplication and overgrowth of these mites, or whether the mites play a role in the pathological changes of rosacea through activation of innate immune system particularly through the cascade of activation and expression of the cathelicidin LL-37.

- **Helicobacter Pylori (HP):**

Several studies have suggested a potential relationship between helicobacter pylori (HP) and rosacea, as it has been shown in some studies that the prevalence of HP infection is higher in patients affected by this condition when compared to the general population.

Rebora et al in 1994 was the first published report of an association of HP with rosacea in a study of 31 rosacea patients that documented positive HP gastritis in 84%, and all the rosacea patients were cleared or improved after treatment with metronidazole (40).

Since that time, there have been many studies that showed the prevalence of HP infection was reported more in rosacea patients compared to healthy controls, and eradicating HP infection in those infected rosacea patients helped to clear the rosacea symptoms particularly in papulopustular rosacea subtype (41 - 45). However, all these studies used different eradication regimens to treat HP, which all contained antibiotics known to be effective in clearing rosacea. In these circumstances, it is difficult to prove whether the rosacea improved because of the eradicating of HP or because of the antibiotic effect. This is an almost universal fault of these studies, together with their small scale.

Others suggest that because HP is so common in the normal population, it is most unlikely that this organism can be related to cutaneous disease. It is certainly the case that no study so far performed is large enough to answer the question fully.

A comparative study, compared the prevalence of HP between rosacea patients and controls, and evaluated the effect of HP eradication. HP was detected by using gastroscopic biopsy in 84% of 50 rosacea patients and 78% of 50 controls. The results showed no significant difference in the prevalence of HP between rosacea

patients and controls. There was no significant improvement of the erythema, and there was only temporary improvement in papulopustules during the 2 weeks of treatment. This was probably a result of the antibiotic regime. So, this study rightly concluded that no significant lessening of rosacea lesions was achieved by eradicating HP infection, and that HP is not related to rosacea (46). In a further double blind controlled study on the effect of eradicating HP in those with both rosacea and HP, showed no benefit was found on an overall rosacea assessment score (47).

Summarising these data, HP is commonly found in patients with rosacea and in the general population. There is no clear evidence of an association, even if a short term improvement of rosacea symptoms is reported after eradication therapy in some patients.

5.5 - Reactive Oxygen Species (ROS):

The reactive oxygen species include superoxide and hydroxyl radicals and other inactivated oxygen forms such as hydrogen peroxide and singlet oxygen. The role of ROS in rosacea has been investigated through the actions of the medications used for treating rosacea. Rosacea treatment including oral tetracyclines (48), topical azelaic acid (49), topical metronidazole (50) and retinoids (51) all inhibit the generation of ROS in neutrophils. This results in low level of ROS and supports the hypothesis that ROS involvement is relevant to rosacea. The level of ROS in the skin was compared in rosacea patients and healthy individuals and the levels were higher in rosacea skin lesions than normal healthy people (52). The UV generates production of ROS which activate cellular signalling in keratinocytes mediating cytokine induction by TNF-α and chemokine production by TLR2 stimuli in monocytes. Also ROS stimulate fibroblasts, actuates matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinases expression. ROS increases MMP-1 and MMP-2 mRNA expression and depress proalpha I and proalpha III (53).

Oztas et al, found decreased activity of the superoxide dismutase (an oxygen radical quenching enzyme) and increased malondialdehyde levels (lipid peroxidation product as a result of free radical activity) in patients with severe rosacea compared with controls (54). Another study investigated plasma ROS activity and antioxidant status and their relationship with the HP infection in 29 rosacea patients. The result showed higher level of malondialdehyde and a lower level of antioxidant in rosacea patients compared with controls but no correlations with HP seropositivity (55).

Therefore, up-regulated activity of the ROS in the skin could result in the inflammation, vascular changes, and collagen degeneration observed in rosacea. However, once again, these studies demonstrate involvement in the process of inflammation in rosacea, but fall short at implying a pathogenic role.

5.7 - Environmental Factors:

It has been proposed that damage to dermal connective tissue often caused by solar irradiation may be the initiating event in rosacea. Sun damage may affect the lymphatic and blood vessel function due to damage to the dermal support network of elastic and collagen fibers. This may result in endothelial damage and leaking of serum containing inflammatory mediators, and metabolic waste, which may cause the telangiectasia, persistent erythema, and skin odema. These finding are usually present in sun-exposed areas of rosacea patients including the face and chest and are usually more common in fair-skinned people.

Many authors believe that rosacea results from the effects of climatic exposures that damage both cutaneous blood vessels and dermal connective tissue. The pivotal role of sunlight is supported by the distribution of the erythema and telangiectasia on the facial convexities and sparing of the sun-protected areas. Also, the reputed flare up of the rosacea in the spring and summer seasons, as well as the presence of solar elastotic changes in the histology of rosacea support this view (56).

The UV exposure may trigger activation of the innate immune and / or neurogenic effects. Ultraviolet B (UVB) has been shown to induce the production of many immunomodulatory cytokines such as IL-1, IL-4, IL6, IL8, IL10, and TNF-α, while Ultraviolet A (UVA) can inhibit synthesis of collagen and modulate the activity of some MMPs involved in the degradation and remodelling of the dermal extracellular matrix (57). Bielenberg et al, showed that UV-B increased the production of VEGF and basic fibroblast growth factor 2 (bVGF-2) by keratinocytes in mice. These cytokines are able to stimulate the proliferation of new blood vessels and increase telangiectasia (58).

Common Triggers of Rosacea Flare-up:

Based on the NRS survey of 400 rosacea patients, the percentages of the most common triggering factors of rosacea are as shown in table 3 (59). While the list of these potential trigger factors range from weather changes, to emotions, to different types of food, nearly all are related to flushing. To help rosacea patients to avoid triggering factors, patients are advised to keep a rosacea trigger diary on a daily basis to observe and record weather conditions, types of food and drinks consumed, facial products used, medications, and any other relevant factors.

Table 3 - Triggering Factors: (59)

No	Trigger	Patient affected %
1	Sun exposure	81%
2	Emotional stress	79%
3	Hot weather	75%
4	Wind	57%
5	Heavy exercise	56%
6	Alcohol	52%
7	Hot baths	51%
8	Cold weather	46%
9	Spicy food	45%
10	Humidity	44%
11	Indoor heat	41%
12	Skin care products	41%
13	Heated beverages	36%
14	Cosmetics	27%
15	Medications	15%
16	Medical conditions	15%
17	Fruits	13%
18	Marinated meats	10%
19	Vegetables	9%
20	Dairy products	8%

5.8 - Medications:

- **Topical Steroids:**

It is well know that oral or topical steroids can induce features resembling rosacea. The prolonged use of potent topical steroids on the face often produces symptoms and signs resembling papulopustular rosacea. If application of steroids continues, fixed erythema and telangiectasia develop and may give similar features to idiopathic rosacea (60). Whenever corticosteroid application stops, rosacea flares up badly, leading to state of dependency. Patients usually do not recognise the cause and on the contrary continue applying steroids as the application produces transient improvement in the symptoms. Even mild topical steroid application for a long period of time can produce rosacea especially in children (61). Long term use of a nasal steroid spray may also provoke steroid rosacea (62).

- **Topical Calcineurin Inhibitors:**

Topical application of calcineurin inhibitors can cause a rosaceiform dermatitis characterized by numerous small papules and pustules with mild erythema on the face. The distribution of this eruption is generally more widely spread than the centrofacial pattern of papulopustular rosacea. Demodex mites have been have found to be abundant in the skin of those patients. This is attributed to the immunomodulating effects of calcineurin inhibitor agents which may act on a number of ways, including through an impact on the numbers of these mites in and on the skin (63).

- **Epidermal Growth Factor Receptor (EGFR) inhibitors:**

There is an increasingly reported development of a papulopustular eruption resembling papulopustular rosacea that results from the use of Epidermal Growth Factor Receptor (EGFR) inhibitors in oncology. This may happen in up to 90% of

patients. It is more similar to papulopustular rosacea than acne vulgaris as it lacks comedones. These inflammatory lesions can involve the skin on the face, scalp, and trunk. The eruption has been attributed to the role of EGFR inhibitors in modifying cytokine signalling in the skin (64). It is reported that this eruption is also associated with an increased density of DF in the affected skin (65).

- **Other Medications:**

Some other drugs such as amiodarone or vaso-dilating drugs (e.g. nifedipine) may affect rosacea through vasodilatation by inducing flushing.

5.9 - Hormonal Changes:

Some authors have noted an increase in rosacea during pregnancy, menses or perimenopausal periods. This has been attributed to the hormonal changes found at these times. There are many case reports of the association of rosacea fulminans and pregnancy (66). Others have reported rosacea fulminans occurring abruptly during pregnancy with no history of preceding disease or any other triggering factors. This may resist treatment and persist during pregnancy. However this case improved and responded to treatment after delivery (67).

Figure 5 - Molecular mechanisms of rosacea pathogenesis :(57)

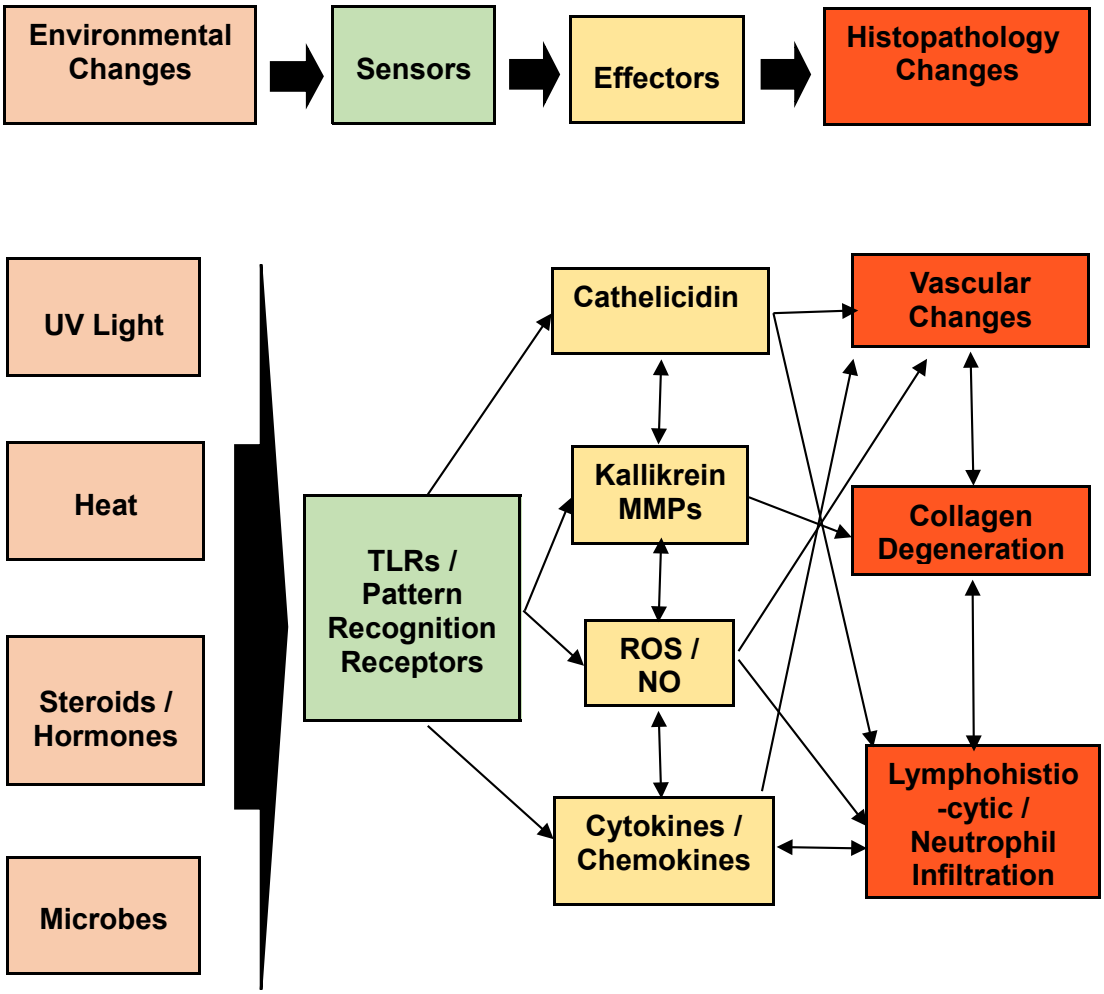


Figure 5 shows summary of molecular mechanisms of rosacea pathogenesis. Environmental changes, hormones and microbes challenges sensed the TLRs and other pattern recognition receptors. TLRs signaling induce effector molecules including cathelicidin, kallikrein, MMP, ROS, NO, cytokines and chemokines. These effectors modify the dermal structure by vascular changes and collagen degeneration accompanied with inflammatory cells recruitment. Infiltrated neutrophils and lymphocytes will be the further source of effector molecules, which activate TLRs directly and indirectly.

6. Diagnosis

To date, no diagnostic test for either cutaneous or ocular rosacea, including any serological or histological markers, has been described. Diagnosis of cutaneous rosacea still depends on the clinical symptoms and signs and history review. The diagnosis may helped by skin biopsy for histology and direct immunofluorescence studies (DIF) to exclude other skin conditions affecting the face such as lupus erythematosus, facial dermatitis, facial sarcoidosis, and other alternative diagnosis.

The diagnosis of ocular rosacea also relies on observation of the eye signs and symptoms including conjunctivitis, foreign body sensations, burning or stinging, dryness, itching, light sensitivity, blurred vision, telangiectases of the conjunctiva and lid margin, lid and periocular erythema, anterior blepharitis or meibomian gland dysfunction.

There have been recent and ongoing studies to try to develop a diagnostic marker which may enable earlier diagnosis and treatment of both skin and eyes in rosacea. These studies are working on the glycomic profile of tears and saliva of rosacea patients, particularly those with ocular rosacea, as a biomarker for this disease. It was demonstrated that certain polysaccharides such as sulfated oligosaccharides are increased in the tears of patients with rosacea. More recently there was a published report on glycomic analysis of tears and saliva, and this confirmed that O-glycans were increased in roseatic tear and saliva samples (68).

Until the development of new diagnostic markers, rosacea is still a clinical diagnosis, which is sometimes confirmed by histological examination and negative DIF study as well as by negative autoantibody screening.

In 2002, the Committee of the National Rosacea Society (NRS) implemented standard clinical diagnostic criteria for rosacea (2). The committee based the

standard classification system on present scientific knowledge and morphologic characteristics. This avoids assumptions on pathogenesis and progression, and provides a framework that can be readily updated and expanded in future as new discoveries are made.

The presence of one or more of the following signs as shown in table 4 with a central facial distribution is indicative of rosacea. These signs are usually transient, and each sign may occur independently. Many patients may present with a number of features at the same time. Rosacea can vary substantially from one individual to another, and in most cases, some rather than all of the signs and symptoms appear.

Table 4 - Diagnostic Criteria of Rosacea: (2)

Diagnosis can be made clinically by presence of one or more of the below primary symptoms and may include one or more of the secondary features:

No	Primary Features	Secondary Features
1	Flushing (Transient Erythema)	Burning or Stinging
2	Non Transient Erythema	Plaque
3	Papules and Pustules	Dry Appearance
4	Telangiectasia	Oedema
5		Ocular Manifestations
6		Peripheral location
7		Phymatous Changes

Table 5 - Primary Features:

All these features can be graded as absent, mild, moderate, or severe.

No	Sign	Features
1	**Flushing (Transient Erythema)**	This is characterized by frequent attacks of flushing. This facial redness is often the earliest sign of the rosacea. Perimenopausal flushing should not be considered significant unless accompanied by other features of rosacea.
2	**Persistent Redness (Non-transient Erythema)**	Persistent facial redness is the most common individual sign of rosacea, and may resemble a blush or sunburn that does not go away.
3	**Papules and Pustules**	Papules are small red raised lumps, which may be associated with pus-filled lesions. Sometimes these papules enlarge and form nodululocystic lesions. These papules may resemble acne, blackheads are absent and burning or stinging may occur. Comedones are not features of rosacea.
4	**Telangiectasia**	These are small blood vessels that become visible on the face. They can present without other features of rosacea in other normal people.

Table 6 - Secondary Features:

These features can present with or without any of the primary features.

No	Sign	Features
1	**Burning or Stinging**	Burning or stinging sensations may often occur on the face. Itching or a feeling of tightness may also develop.
2	**Dry Appearance**	The central facial skin may be rough and dry and may resemble eczema. This is sometimes associated with features of seborrheic dermatitis.
3	**Plaques**	Raised red patches may develop as a result of confluent areas of inflammation without changes in the surrounding skin.
4	**Skin Thickening - Rhinophyma**	Skin may thicken and enlarge due to a variable combination of fibrosis, sebaceous hyperplasia and lymphoedema. The areas most affected are the nose. Other areas that can be affected by similar changes are the forehead, chin, eyelids and ears.
5	**Oedema**	This is facial swelling, and can affect parts of the face such as periorbital or glabellar areas. It can be recurrent or chronic persistent, may accompany other signs of rosacea or occur independently.
6	**Eye Irritation**	Ocular manifestations of rosacea are common. These may include itching, burning, stinging sensations and light sensitivity. The eyelids also may become red and swollen. Other features are Chalazia and styes. Severe cases can result in corneal damage.

6.3 - Histological Features:

The histological features are predominantly dermal changes and reflect the clinical subtypes. There is usually evidence of vascular and lymphatic channel dilatation with some solar elastosis. There is a mixed inflammatory infiltrate of lymphocytes and neutrophils and macrophages, which can be perifollicular, interfollicular and perivascular as shown in figure 6 and 7. Some lesions show granulomatous features. Demodex mites are often found in the follicle as seen in figure 7 (69).

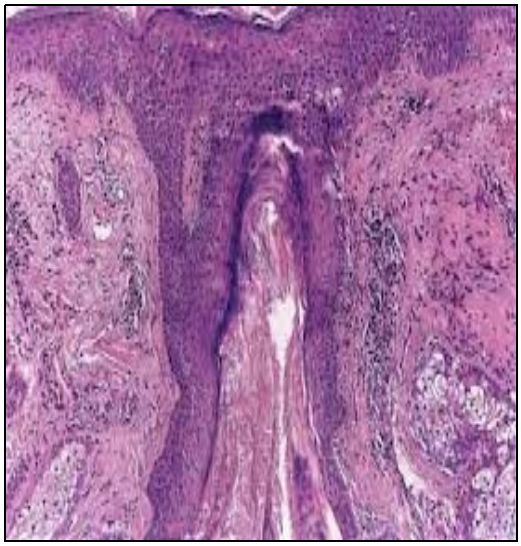


Figure 6 - Rosacea Histology

H&E stain, X40

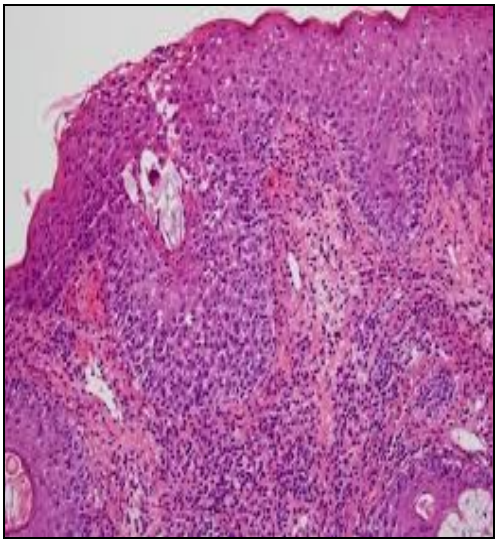


Figure 7 - Rosacea Histology

H&E stain, X40

7. Classification

The first classification and staging of rosacea was developed by the NRS Experts Committee and was published in April 2002 issue of the Journal of American Academy of Dermatology (2).

The committee developed this standard classification system alongside the diagnostic criteria to use as a diagnostic instrument in investigating the manifestations and relationships of the subtypes and variants of rosacea.

The standard description of groups of rosacea is important in research studies to ensure that data are comparable. The classification system also helps in communication between different clinical and scientific researchers, including dermatologists, primary care physicians, ophthalmologists and other specialists such as health and insurance administrators, patients and the general public.

There are four subtypes of rosacea, defined as common patterns or groupings of signs and symptoms and one other variant as shown in table 7.

Table 7 - Rosacea Subtypes: (2)

Main Rosacea Subtypes		
No	**Subtype (Name)**	**Clinical Features**
1	**Erythematotelangiectatic Rosacea - (ETR)**	Persistent central facial erythema, frequent flushing, may be telangiectasia.
2	**Papulopustular Rosacea - (PPR)**	Red dome shaped papules and pustules with back ground erythema.
3	**Phymatous Rosacea - (PR)**	Tissue hypertrophy involving different areas of the face but commonly affect the nose.
4	**Ocular Rosacea - (OR)**	Different ocular inflammation of eye lid, conjunctiva, meibomian gland, and cornea.
Rosacea Variant		
1	**Granulomatous Rosacea - (GR)**	Chronic inflammatory facial eruption of dome shaped uniform hard, brown, yellow, or red papules or nodules with granulomatous features histologically.

7.1 - Subtype 1 - Erythematotelangiectatic Rosacea (ETR):

Characterized by persistent central facial erythema, with a tendency to repeated and prolonged episodes of flushing. This may or may not be associated with visible blood vessels. Other features may include stinging and burning sensations or mild facial oedema. Patients typically have skin type I or II and usually present with photodamage in these areas. This is considered to be an important element both in production and exacerbation of ETR. In addition, patients have sensitive, easily irritated skin that is abnormally reactive. Stinging and burning sensations develop easily to different stimulants such as facial products, air fresheners, and others. The skin of these patients can be rough, slightly eczematous and this is sometimes referred as rosacea dermatitis (2).

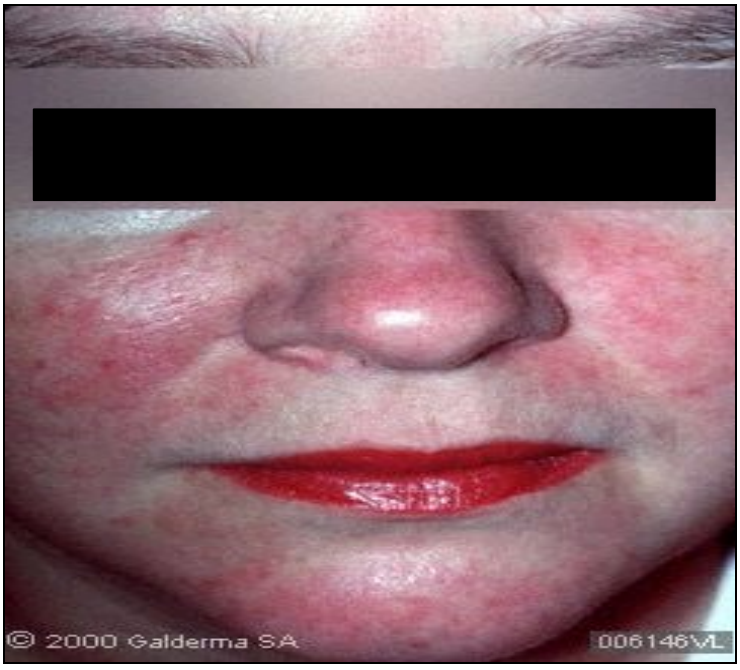


Figure 8 - Erythematotelangiectatic Rosacea (ETR)

ETR can be difficult to distinguish clinically from heliodermatitis (Farmers face or Fisherman's face), a skin condition that affects outdoor fair skinned workers (skin type 1 or 2), and result from prolonged exposure to sun. Those people present with persistent facial and neck erythema with or without telangiectasia (70).

7.2 - Subtype 2 - Papulopustular Rosacea (PPR):

This is also known as classic rosacea and is characterized by persistent facial redness with transient papules or pustules or both in various stages of evolution. The episodes of inflammation may lead to chronic oedema. Some patients present with recurrent facial flushing that can precede the appearance of inflammatory papulopustular lesions. This subtype may resemble acne vulgaris, and was called acne rosacea. However, there are no comedones unless in some situations where rosacea can coexist concomitantly with acne.

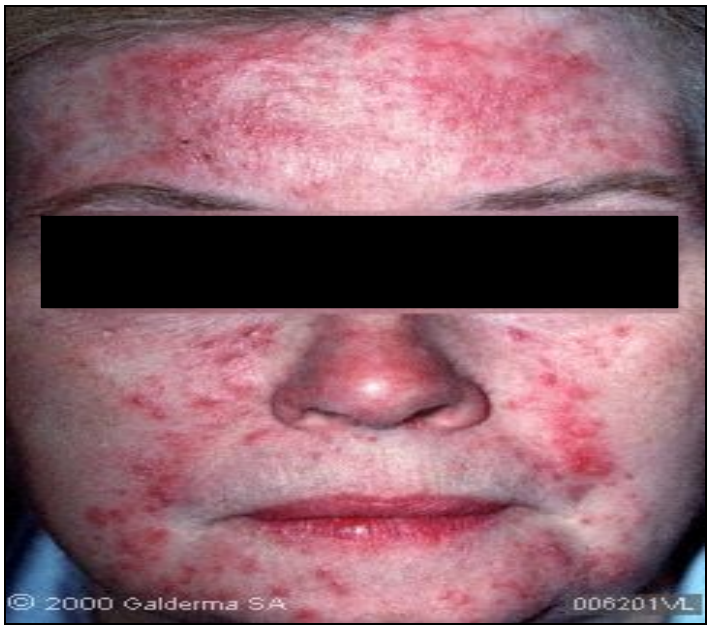


Figure 9 - Papulopustular Rosacea (PPR)

Male bald patients may develop papulopustular inflammatory lesions on the bald scalp area in continuity with the facial eruption. Extra facial PPR has been described on the trunk and abdomen which can be difficult to distinguish from folliculitis (71). It may present in combination with ETR or ocular rosacea. The condition waxes and wanes, sometimes appearing to go into partial remission leaving no scars and at other times becoming active and inflammatory for no apparent reasons.

7.3 - Subtype 3 - Phymatous Rosacea (PR):

This subtype reflects the hypertrophy of sebaceous glands in nasal skin, and occasionally elsewhere. It is characterized by persistent, firm, non-painful, non-pitting skin thickening with irregular surface nodularities which result in an enlargement of the nose from excess tissue (Rhinophyma). The earliest clinical manifestation of rhinophyma is the appearance of dilated pores (Patulous Follicles) with subsequent development of telangiectatic vessels on the end and sides of the nose. It can occur with other subtypes of rosacea. However, it may also occur in patients with acne vulgaris and occasionally result from chronic actinic damage.

Rhinophyma is sometimes considered as the end stage of rosacea. However, it can occur in patients with very mild rosacea changes or even with no evidence of the disease at all. There is no relation between the development of the phymatous changes and the severity, duration or subtypes of rosacea.

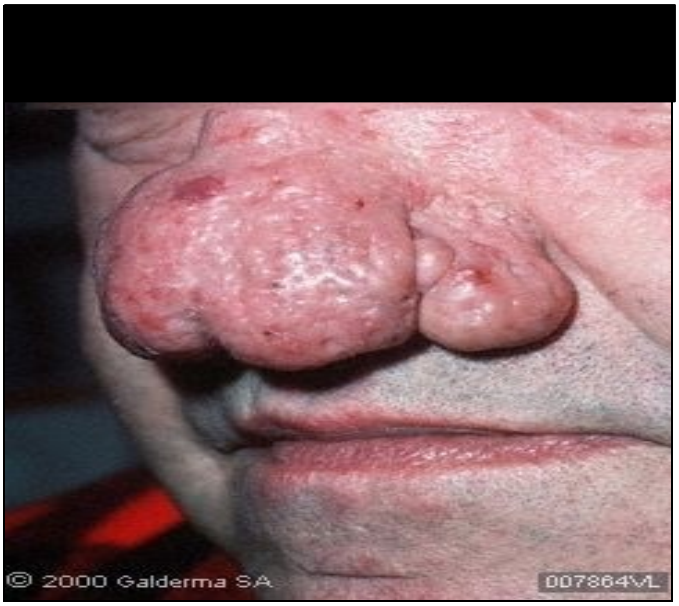


Figure 10 - Phymatous Rosacea (PR)

Other areas may develop the same changes of rhinophyma; this commonly affects male patients and examples are in table 8.

Table 8 - Other forms of Phyma according to site involved: (72)

No	Site of Phyma	Features
1	**Forehead / (Mentophyma)**	Cushion like firm swelling of the central forehead
2	**Chin / (Gnathophyma)**	Rare, affects mainly the central chin, give rise to asymmetrical swelling.
3	**Eyelids / (Blepharophyma)**	Swelling of the eye lids, usually as a component of oedematous rosacea, but also can happen in severe papulopustular or ocular rosacea.
4	**Ears / (Otophyma)**	Affects the lower half of the helices and lobes of ears.

7.4 - Subtype 4 - Ocular Rosacea (OR):

This is defined as a range of changes that occur either in the eye lid, eye lashes, or eyes of patients with rosacea. The causes for these changes are unknown. It can precede rosacea of the skin, so diagnosis can be very difficult. Those with erythematotelangiectatic and particularly papulopustular rosacea appear to be particularly vulnerable to develop ocular inflammation with up to 50% of patients affected (2).

Symptoms are non-specific and include itching, tearing, dryness, gritty sensations, crusting of the eye lids, irritation to the eye lenses, and recurrent styes. Also light sensitivity and sometimes blurred vision may occur. Potential visual loss may result from corneal damage. Most patients do not volunteer these symptoms unless specifically asked.

Patients with PPR appear to be more prone to developing OR. The duration or severity of ocular rosacea dose not relate to the duration or severity of the skin disease, however it has been suggested that ocular rosacea can be correlated to the tendency of patients to flush (73). Another study found a significant relationship between ocular involvement and the severity of telangiectasia (74).

One presentation of OR is chronic conjunctivitis which is characterized by inter-palpebral bulbar conjunctival hyperemia, as well as a chronic papillary reaction (75). Akpek et al, described cicatricial conjunctivitis involving the lower eye lid as one of the most common ocular findings in rosacea (76). Other reports described chronic cicatrizing conjunctivitis affecting the upper eyelids, similar to the classical findings in trachoma (77). Pinguecula and conjunctival fibrosis have also been reported in up to 20% of patients (78).

The summary of the commonest manifestations of OR according to the site of the eye involved are shown in table 9.

Table 9 - Commonest manifestations of ocular rosacea: (1)

No	Site of eye involvement	Complications / Features
1	**Eye Lids**	Blepharitis, lid telangiectasias.
2	**Conjunctiva**	Conjunctivitis, conjunctival injection, or overgrowth.
3	**Meibomian Glands**	Chalazion, hordeolum internum.
4	**Lacrimal Glands**	Reduced tear secretion.
5	**Cornea**	Punctuate erosions, keratitis, and perforations.
6	**Sclera**	Scleritis, episcleritis.
7	**Uvea**	Uveitis.
8	**Iris**	Iritis.

7.5 - Relationship of Rosacea Subtypes to each other:

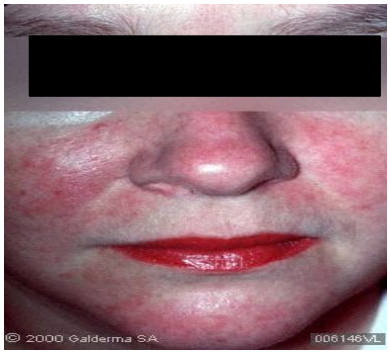
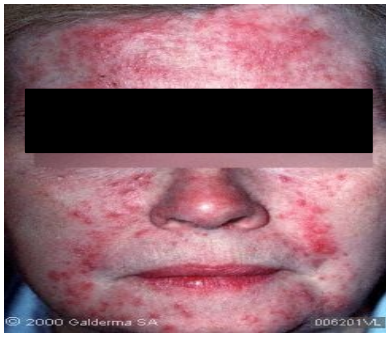
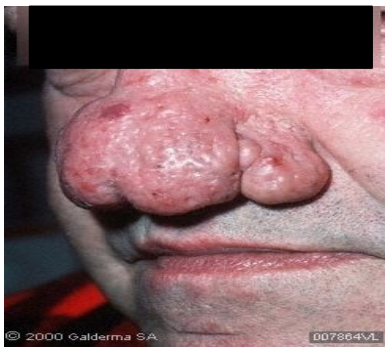
Many patients experience characteristics of more than one subtype of rosacea at the same time, others may develop the subtypes in succession. While rosacea may or may not evolve from one subtype to another, each individual sign or symptom may progress from mild to moderate to severe. Early diagnosis and planning long term management including patient education and avoiding of any triggering factors are therefore recommended.

In one of the NRS surveys of 1231 rosacea patients showed that 83% of the respondents had ETR, 62% had PPR, 50% had OR and 15% had PR. The survey showed that the general trend of the rosacea is to progress from subtype 1 (ETR) to subtype 2 (PPR) and then some cases to subtype 3 (PR). However, subtype 4 (OR) may develop at any stage even before skin symptoms (79).

A further study of 135 rosacea patients showed that PR was more frequently associated with ETR than PPR ($p < 0.001$). 66% of patients developed ETR before PPR, 92% developed ETR before PR and 83% developed PPR before PR. The majority of patients developed cutaneous rosacea associated features before ocular rosacea (80).

In conclusion, significant differences exist between the subtypes of rosacea. There are no accurate data indicating the risk of the rates of progression of one subtype of rosacea to another.

Table 10 - Summary of Rosacea Subtypes: (2)

Rosacea Subtype	Signs and Symptoms	%
Subtype I : Vascular Erythemato-telangiectatic (ETR)	- Flushing - Redness (erythema) - Telangiectasia 	55% - 70%
Subtype II : Inflammatory Papulopustular (PPR)	- Redness (erythema) - Papules, pustules 	25% - 40%
Subtype III : Phymatous (PR)	- Thick skin, nodules - Irregular skin surface - Enlargement of nose 	5%
Subtype IV : Ocular Rosacea (OR)	- Foreign body sensation - Burning and stinging - Dryness and itching - Ocular photosensitivity - Blurred vision 	3% - 50%

7.6 - Granulomatous Rosacea (GR):

Granulomatous rosacea (GR), considered as a distinct variant of rosacea characterized by non caseating epitheloid cell granulomas histologically. It is a rare condition characterized clinically by an eruption of hard reddish papules or small nodules, occurring on a thickened indurated erythematous base as shown in figure 11. It may be severe and can lead to scarring. These lesions tend to be less inflammatory than papules and pustules and can vary in size among patients but are monomorphic in each individual patient. The eyelids, lower part of the forehead, nasolabial folds, cheeks and perioral area are frequent sites of involvement. The course is chronic and unremitting (81).

Figure 11 - Granulomatous Rosacea (GR)

GR may occur in locations other than those in which the phymas are observed. The presence of other physical signs of rosacea is not needed for a diagnosis of the GR. Although the exact etiopathogenesis of GR is unknown, the role of a delayed hypersensitivity reaction against keratinized cells, pilosebaceous structures and microbial organisms has been suggested (82). Treatment of GR is not different from that of classical rosacea and so the use of oral antibiotics such as tetracycline, doxycycline, or minocycline is usually effective (83). Nevertheless, a tendency to relapse or recur may persist for several years.

8. Other Rosacea Variants

There are other similar skin conditions considered as variants of rosacea reported in the literature. However, many authors believe these variants may not be related to rosacea as they may have different histology and may have a different pathogenesis. The NRS committee recognizes these conditions as separate entities and concluded that there is insufficient basis at present to include the following conditions as subtypes or variants of rosacea (2).

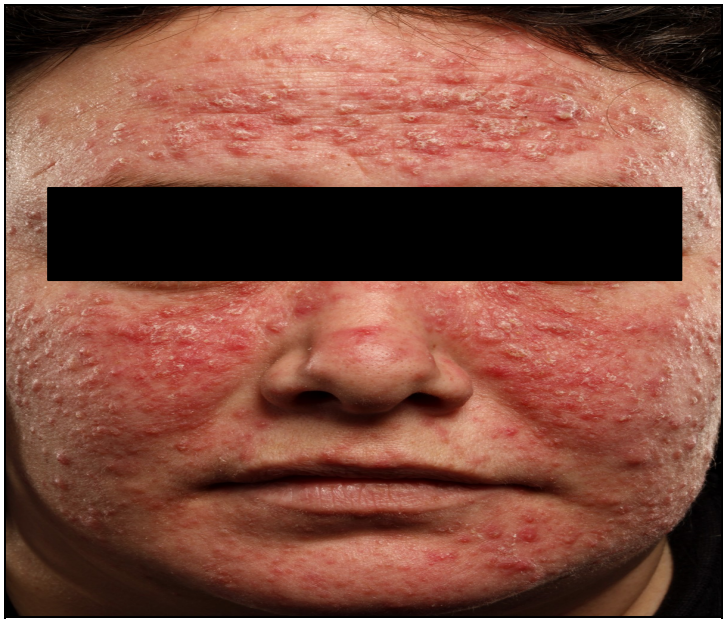
8.1 - Rosacea Conglobata / Rosacea Fulminans (Pyoderma Faciale):

This is a dramatic development of large facial nodulo-cystic lesions with marked erythema predominantly occurring in young women. In the past this was labelled as pyoderma faciale and suggested to be related to acne vulgaris. This has been renamed recently as rosacea conglobata / rosacea fulminans because of the association with marked redness and erythema. However, this condition can lead to devastating cosmetic scarring which is unlike other rosacea subtypes (2).

The condition is characterized by sudden generalized facial pustular lesions with erythema and facial oedema in patients who have suffered from frequent flushing and sensitive skin but without any other clinical features of rosacea or acne vulgaris. There is usually no preceding history of acne or rosacea. Systemic symptoms may occur, along with raised inflammatory markers (84). It can be present in localised forms affecting the cheeks, jaw line or chin. It has been reported to be associated with pregnancy, suggesting hormonal factors may play a role as mentioned in the aetiology of rosacea in the previous section.

Treatment is usually with systemic antibiotics. However, because of the risk of scarring, associated systemic symptoms and raised inflammatory markers, It is been reported to be most successfully treated with systemic steroids and oral isotretinoin.

In one series, 10 out of 20 patients were treated with oral prednisolone at 1 mg/kg/day combined with oral isotretinoin 0.2 – 0.5 mg/kg/day. The prednisolone was tapered off over 2-3 weeks and the isotretinoin continued for 3 -4 months (85).



Figures 12 - Rosacea Fulminans

Figure 12, is the photo of young lady patient who presented to my clinic in 2011 at Doncaster Royal Infirmary Hospital, with history of sudden onset of diffuse red nodules and pustules with some erythema and skin oedema on the face. There was no history of acne, rosacea or flushing in the past and no obvious triggering factors. She was diagnosed as rosacea fulminans after skin biopsies for histology and DIF showed histological features of rosacea with negative DIF and negative ANA blood test with raised inflammatory markers, as well as negative skin culture. She was treated with a tapering dose of oral prednisolone 20mg daily for 2 weeks with oral isotretnoin 0.5mg/kg daily for 4 months with a very good result.

8.2 - Steroid Induced Rosacea:

The excessive, regular use of topical fluorinated steroids on the face often produces an array of skin complications, including an eruption clinically indistinguishable from rosacea called 'steroid-induced rosacea' or ‘latrosacea’ (86). Steroid-induced rosacea is characterized by centrofacial, perioral, and periocular monomorphic inflammatory papules and pustules distributed in areas that have been chronically exposed to topical steroids, especially of fluorinated type. The appearance is of a flaming red, scaly, papule covered face (red face syndrome) (87).

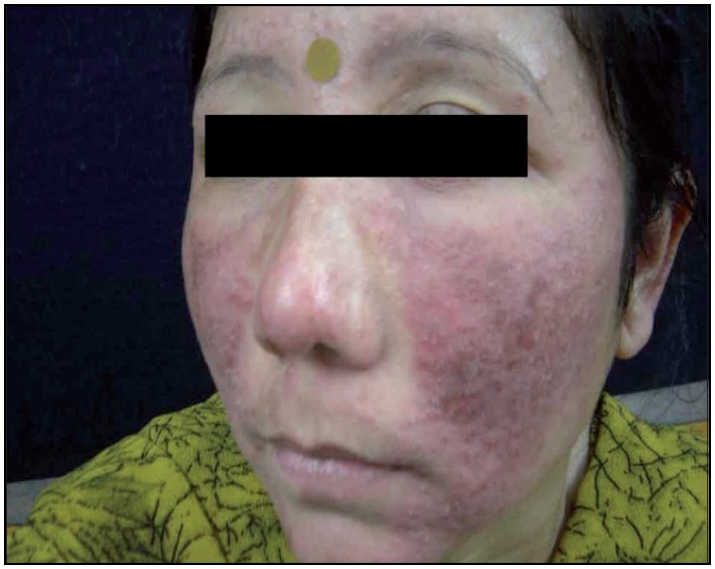


Figure 13 - Steroid Induced Rosacea

Continued or overuse of topical steroids can result in thinning of the skin as well as skin dependency on the steroid. At first the vasoconstrictive and anti-inflammatory effects of the steroids result in what seems to be clearance of the primary dermatitis but persistent use leads to epidermal atrophy, degeneration of dermal structure and collagen deterioration after several months (88). Also steroids inhibit the release of a natural vasodilator called endothelium-derived relaxing factor. Prolonged used of

topical steroids leads to vasoconstriction which in turn leads to the build-up of multiple metabolites such as nitric oxide, a potent vasodilator. Once the steroid is discontinued, the vasoconstrictor effect ceases and the diameter of the blood vessels is enlarged beyond their original pre-steroid diameter because of the accumulation of the nitric oxide, which in turn exacerbates the erythema, burning sensation and the pruritus (89).

Treatment involves discontinuation of the steroid and administration of oral tetracycline or macrolides and non-steroidal topical preparations. Once therapy is begun, clearing of the lesions may take several months.

9. Differential Diagnoses

There are many other skin diseases which can resemble rosacea and occasionally it is difficult to differentiate between them. Investigations including skin biopsy for histology or DIF, skin allergy tests and additional blood tests may be needed to exclude other differential diagnosis.

Differential diagnosis of rosacea depends on the subtypes of rosacea as shown in tables 11, 12, 13 and these include: (1)

Table 11 - Differential Diagnosis of ETR:

No	Differential diagnosis	How to differentiate it from ETR
1	Disorder of flushing	History, clinical examination, blood test.
2	Lupus erythematosus	History, clinical examination, blood test, skin biopsy.
3	Seborrheic eczema	History, clinical examination.
4	Atopic dermatitis	History, clinical examination.
5	Contact and photo contact dermatitis	History, clinical examination, patch test, photo patch test
6	Facial sarcoid	History, clinical examination, blood test, skin biopsy.
7	Topical steroid misuse	History, clinical examination.
8	Facial erysipelas	History, clinical examination.
9	Jessner's lymphocytic infiltration	History, clinical examination, skin biopsy.
10	Polymorphic light eruption	History, clinical examination.
11	Dermatomyositis	History, clinical examination, skin biopsy, blood test.

Table 12 - Differential Diagnosis of PPR:

No	Differential diagnosis	How to differentiate it from PPR
1	Acne vulgaris	History, clinical examination.
2	Acne agminata	History, clinical examination, skin biopsy
3	Perioral dermatitis	History, clinical examination.
4	Seborrheic eczema	History, clinical examination.
5	Facial sarcoid	History, clinical examination, skin biopsy, blood test.
6	Tinea faceii / candida	History, clinical examination, skin scraping / swabs.
7	Pityriasis folliculorum	History, clinical examination, skin scraping.
8	Jessner's lymphocytic infiltration	History, clinical examination, skin biopsy.
9	Polymorphic light eruption	History, clinical examination.

Table 13 - Differential Diagnosis of PR:

No	Differential diagnosis	How to differentiate it from PR
1	Lupus pernio (Sarcoid)	History, clinical examination, skin biopsy, blood test.
2	Lupus erythematosis	History, clinical examination, skin biopsy, blood test.
3	Lupus vulgaris	History, clinical examination, skin biopsy, blood test.
4	BCC / SCC/ Lymphoma	History, clinical examination, skin biopsy.
5	Angiosarcoma	History, clinical examination, skin biopsy.
6	Acrocyanosis	History, clinical examination.

9.1 - Lupus Erythematosus (LE):

Lupus erythematosus (LE) is a heterogeneous connective-tissue disease associated with polyclonal B-cell activation and is believed to result from the interplay of genetic, environmental, and hormonal factors. LE is more common in women than men especially the systemic variety by at least 6 to 1. It also varies between different ethnicities. For example, the prevalence of SLE in African American women is 4 in 1000 compared to Caucasian American women at 1 in 1000 (90).

The spectrum of lupus disease involvement can vary from limited cutaneous involvement to devastating systemic disease. Cutaneous lupus erythematosus (CLE) is the second most common presenting symptom of autoimmune LE. Lesions precede the onset of systemic symptoms in 25% of patients, many of whom present to dermatologists for their initial evaluation (91).

The main subtypes of cutaneous lupus (CLE) according to the James Gilliam classification, based on the presence of interface dermatitis, are acute cutaneous (ACLE), subacute cutaneous (SCLE) and chronic cutaneous (CCLE) (92). Further subdivisions of CCLE include discoid LE (DLE) and other atypical LE specific lesions, including chilblain LE, LE tumidus (LET), and LE panniculitis.

ACLE accounts for about 6% of patients with CLE and it is characterized by the classic “butterfly rash” overlying the malar cheeks and nose as in figure 14. The rash is photo-aggravated and strongly associated with exacerbations of SLE. Lesions typically resolve without atrophic scarring although areas of post-inflammatory dyspigmentation may persist (93).

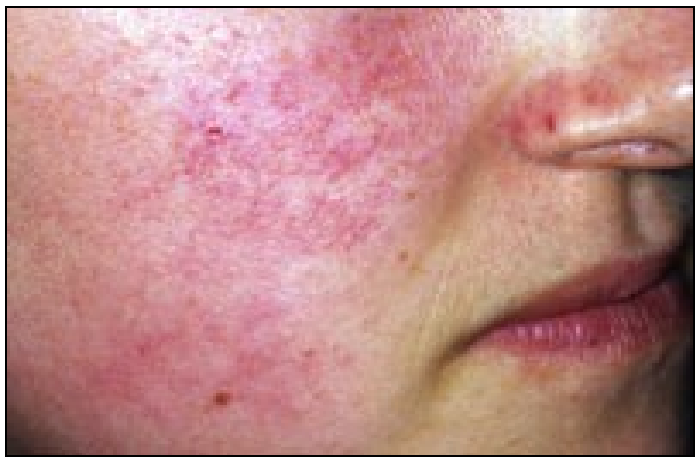


Figure 14 - Butterfly rash of Lupus Erythematosus

DLE is the most common form of CCLE and classically presents as erythematous, coin-shaped plaques with central hyperkeratosis as in figure 15, with 70% of cases limited to the head and scalp. This pattern is rarely associated with systemic disease (94).

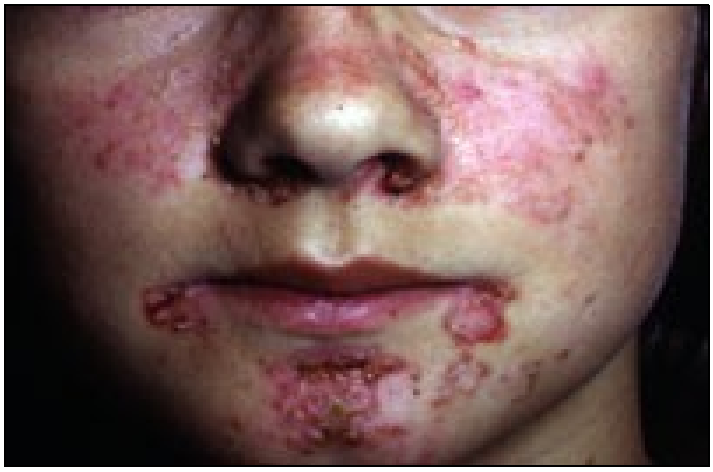


Figure 15 - Discoid Lupus Erythematosus

SCLE is characterised by a photosensitive rash in up to 85% of patients and the lesions are mainly located to sun exposed areas; neck, chest, upper back, shoulders, dorsal parts of the arms and hands but surprisingly the face and scalp are seldom involved. The lesions start as erythematosus plaques or papules and then

become either widespread annular, polycyclic lesions that clear centrally or papulosquamous (psoriasiform) lesions or rarely a combination of these two forms as in figure 16. The lesions are non-scarring but often heal with pigmentary changes that are long lasting.

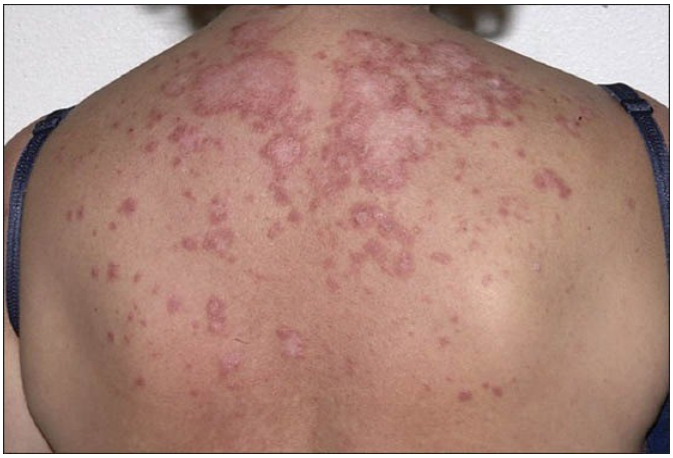


Figure 16 - Subacute Cutaneous Lupus Erythematosus

A diagnosis of chronic cutaneous lupus is made based on the clinical findings of photosensitivity, erythema, follicular plugging, dyspigmentation, telangiectasia, and skin atrophy. Scarring and skin atrophy are characteristic of DLE. The diagnosis is supported by skin biopsy for histology and DIF studies as well as the detection of autoantibodies in the blood in some patients. SCLE is strongly associated with the presence of anti-Ro/SSA antibodies, found in about 70% of patients, and positive ANA antibodies in 60 - 80%, whilst between 30 - 50% display the anti-La/SSB antibody which is almost always seen together with the anti-Ro/SSA antibody. In DLE the involvement of autoantibodies is less clear but up to 50% may display low titres of ANA (95).

The histological features of cutaneous lupus depend on the subtype to a degree; however, an overlap in histological findings can occur between the various clinical phenotypes, particularly ACLE, SCLE and DLE. The most characteristic features of cutaneous lupus are an interface dermatitis with vacuolar change of the basal

keratinocytes (vacuolar degeneration or hydropic changes) and lymphohistiocytic inflammatory infiltrates in the early stages as shown in figure 17. However, in the late chronic stage of those phenotypes that resolve with scarring, thickening of the basement membrane and dermal fibrosis and scarring are characteristic features as shown in figure 18. In DLE, periadnexal inflammation, follicular plugging and scaring are common features, whilst in SCLE, epidermal changes and superficial lymphocytic infiltrates are more common. In contrast to DLE lesions, SCLE lesions tend to have little or no hyperkeratosis, basement membrane thickening, periadnexal infiltrate, follicular plugging, deep dermal infiltrate, or scarring (96). Despite these reported differences, blind assessment of histology from different sub-types of LE by experienced skin pathologists is usually unable to accurately diagnose the sub-type.

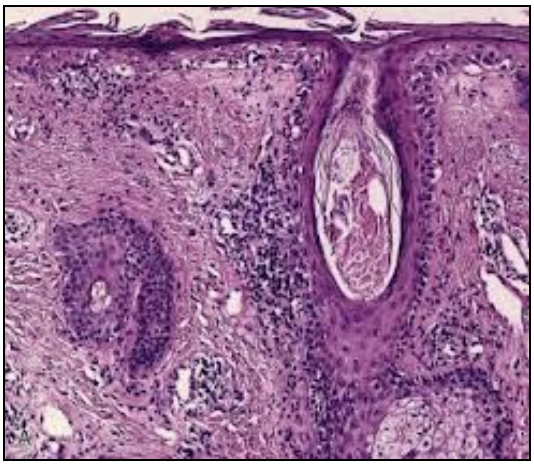


Figure 17 - Lupus Histology

H&E stain, X40

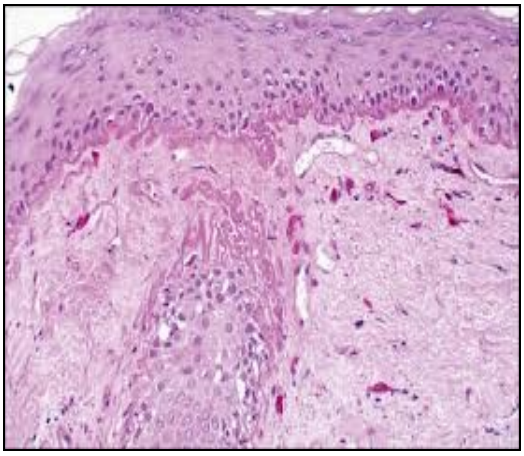


Figure 18 - Lupus Histology

H&E stain, X40

The most characteristic DIF finding from a biopsy of lesional skin is linear or granular deposition of the IgG and/or IgM antibodies at the dermo-epidermal junction and around the hair follicles as shown in figure 19, with or without deposits of complement proteins. DIF performed on normal appearing skin can also show the presence of antibody deposits at the dermo-epidermal junction in SLE, but not in CCLE. When this examination is performed on non-light exposed skin, this is known

as the lupus band test as shown in figure 20. In general, a positive DIF test supports the diagnosis of cutaneous lupus; however, a negative test does not exclude the diagnosis (97). Patients may also have circulating positive autoantibodies in the blood including ANA, and anti-DNA as well as anti-Ro and anti-La antibodies directed against soluble cytoplasm antigens.

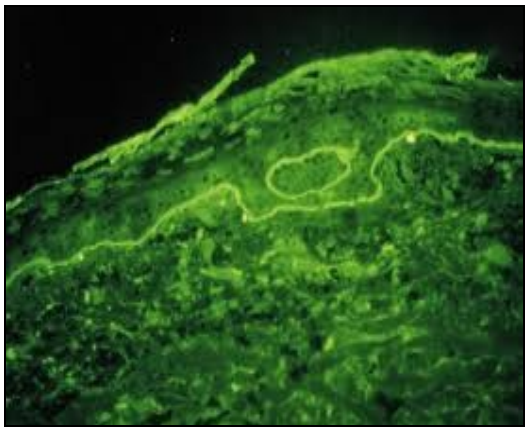


Figure 19 - Linear Deposition of IgG

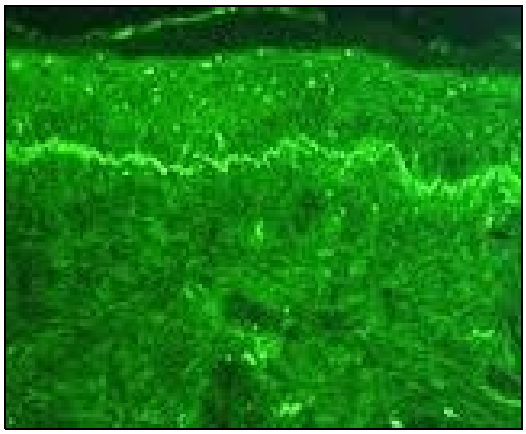


Figure 20 - Lupus band in DIF

All subtypes of cutaneous Lupus may present with facial rashes with varying degrees of erythema and papulo-nodular lesions, and so all fall into the differential diagnosis of rosacea. The butterfly erythema precipitated by UV and sparing those areas with limited UV exposure may be mistaken for ETR, because of the distribution, although pustulation would only be seen with the papulo-pustular version of rosacea. In the chronic stage of lupus, especially with the long term use of potent topical steroids, telangiectasia and erythema can develop in addition to the lupus rash. However, scarring, follicular plugging and skin atrophy are features of discoid lupus and not rosacea. There is, however, a version of discoid lupus that is papular, not pustular in nature, and known as rosaceous lupus, and this is easily confused with rosacea (98). Nevertheless, it is easy to see therefore that a patient with a facial rash due to rosacea, a positive ANA with titre at 1:80 and joint symptoms due to degenerative disease or fibromyalgia might be misdiagnosed as suffering with lupus.

As in rosacea the lupus pathogenesis involved the expression of LL-37. It is been proposed that LL-37 can complex with self DNA activating dendritic cells to contribute to the pathogenesis of lupus erythematosus (99). In one study investigating the relationship of LL-37 in skin of SLE and their role in SLE pathogenesis, skin biopsies were taken from 9 active SLE patients and compared with 6 healthy controls. The expression of LL-37 was significantly higher in the skin of the SLE group than healthy controls (detected by immunohistochemical technique and in situ hybridization, $p<0.001$) (100). An other study determined the expression of several AMPs including cathelicidin LL-37 in 47 patients of different subtypes of CLE compared to 15 healthy controls by analysing the skin lesions for gene and protein expression using real time reverse transcriptase PCR and immunohistochemistry. The results showed LL-37 was significantly more highly expressed in CLE as compared to healthy controls and this is much higher in SCLE than in DLE and LE Tumidus (101).

So, from the above studies, this is may explain partly why rosacea and lupus may both be triggered by UV light and why the skin mostly involved is the sun exposed areas. This is could be the result of the activation and expression of the cathelicidin LL-37 by UV light, although there is also a role for the Ro antibody in patients with SCLE.

Treatment of cutaneous lupus is completely different from that in rosacea, so it is important to confirm a diagnosis before starting treatments. Indeed some treatments for rosacea such as tetracyclines and particularly minocycline may aggravate lupus, and treatment of lupus such as topical or oral steroids may aggravate rosacea. Finally, it is also possible for patients to have both lupus and rosacea at the same time.

10. Management of Rosacea

Rosacea requires long-term treatment. There are many treatment modalities and these treatments depend on stage and severity of the disease. These treatment modalities include topical, oral, laser, and surgical therapies.

10.1 - Aims of the treatment are:

1. Reduce signs and symptoms including skin irritations, stinging, erythema, inflamed papules and pustules.

2. Delay or prevent development progress of the disease from the milder stage to the more sever stage.

3. Facilitate remission and control exacerbation.

4. Maintain skin integrity

5. Improve patient quality of life.

10.2 - Topical Treatments:

These include Azelaic Acid (15%, 20%), Erythromycin (2%), Metronidazole (0.75%, 1%) or Sodium Sulfacetamide 10% + Sulfur 5%. Vehicle selection for topical treatment of rosacea is important as most patients have sensitive skin: the choice between lotion, gel, cream or foam as the delivery system is important as it will affect treatment usage and inevitably influence outcome.

Topical Metronidazole:

It is the most widely used topical treatment in rosacea and available in different forms and concentrations including 0.75% gel, lotion, and cream for twice a day and 1% gel or cream for once daily use. A number of trials have demonstrated it is more effective when compared to placebo (102). there was no statistically significant difference between 0.75% gel or 1% cream with respect to reduction of erythema, papules and pustules, (103).

Topical Azelaic Acid:

A study showed significant improvement of rosacea inflammatory papules and pustules with 15% azelaic acid gel (104). An other study has confirmed that there is no significant difference between once daily and twice daily use of 15% gel (105).

Topical Sodium Sulfacetamide 10% and Sulfur 5% in combination:

This combination of sodium sulfacetamide and sulfur has been used for treatment of rosacea. The mechanism of action is not well known but the sulfacetamide has antibacterial effect and the sulfur has antifungal, anti-demodectic and keratolytic effects. In a double blind placebo controlled study, the inflammatory lesions and the erythema improved significantly in the treated group compared to the placebo group (106).

Topical Erythromycin and Clindamycin

Both are not very commonly used in rosacea however, topical erythromycin has been reported to reduce the erythema, papules and pustules in mild rosacea patients (107). Topical clindamycin twice daily produced clearance similar to oral tetracycline 1000 mg daily for 3 weeks followed by 500mg daily for further 9 weeks (108).

Topical Tacrolimus:

Topical tacrolimus was reported to be effective treatment for rosacea induced by steroids (109).

10.3 - Systemic Treatments:

Systemic treatment is mainly for moderate to severe papulo-pustular or ocular rosacea, and this includes the oral tetracyclines: Tetracycline, Oxytetracycline, Doxycycline, Minocycline, macrolide derivatives such as Erythromycin, Metronidazole, and occasionally drugs such as Dapsone, as well as oral retinoids.

Oral Tetracyclines:

Tetracycline compounds were the first systemic drugs used in the treatment of rosacea, and have been the mainstay of oral therapeutics in this disease for more than 40 years (110). These agents possess anti-angiogenic and anti-inflammatory properties that make them the drugs of first choice in the treatment of rosacea. Several studies have shown that tetracylines, including doxycycline, have immunomodulating properties (111). These anti-inflammatory effects have been used to target several pathophysiological mechanisms in rosacea.

Oral Low Dose Doxycycline:

Low dose doxycycline 40mg (30mg immediate release and 10mg delayed release) once daily is the new trend in rosacea treatment; it provides a sub-antimicrobial dose

that reduces inflammatory lesions without risking an increase in bacterial resistance (112). Efficacy has been demonstrated in a number of trials, including those in which it was compared with placebo (113), conventional dosage of doxycycline 100mg daily (114) and as an adjunct to topical therapy (115), (116), also assessment of the effectiveness and safety (117), and as an effective promoter of quality of life (118).

Oral Macrolides:

Oral erythromycin at a dose of 250mg to 1000mg a day is considered an effective treatment for papulo-pustular rosacea (119). It is usually used when the tetracyclines group is not working or when it is contraindicated such as in the treatment of rosacea in pregnancy or lactation and children of less than 12 years. The use of second generation of macrolides in rosacea including clarithromycin and azithromycin is less common, but they are of proven efficacy (120), (121).

Oral Metronidazole:

Metronidazole is an effective alternative treatment, as demonstrated by Pye and Burton in 1976 (122) at least for inflammatory rosacea. A second study demonstrated equivalence with tetracycline (123).

Oral Isotretinoin:

Multiple reports from the 1980s established the effectiveness of oral isotretinoin for rosacea. Importantly, recalcitrant cases of rosacea have been successfully treated with oral isotretinoin using a dosage range of 0.5 to 1mg/kg/day (124), (125). Isotretinoin also has been reported to reduce nasal size in rhinophyma (126).

10.4 - Laser and Light Treatments:

Vascular laser therapy for telangiectatic rosacea started in the early 1980, and since then it has progressed to include the use of many devices and different types of light producing machines. Recently the approaches of treating rosacea with laser have extended not only to include the treatment of telangiectasia but also now involve remodelling of the dystrophic dermal connective tissue and strengthening the epidermal barrier. Some modalities such as intense pulsed light have also been used to treat inflammatory rosacea. The mode of action of non-ablative laser and light therapy in rosacea is probably through the impact of thermal induced fibroblast and endothelial proliferation leading to cytokine, growth factor and heat shock protein activation.

Vascular Laser:

Laser is used for the treatment of telangiectasia and erythema: the main modalities used are the pulse dye laser (585, 595 nm), the long pulsed dye laser (595 nm), the potassium titanyl-phosphate lasers (532 nm) and diode laser (532 nm). A number of studies have demonstrated effectiveness using the pulsed dye laser (127), and the KTP laser (128). The use of pulsed dye laser results in a significant improvement in quality of life in ETR patients (129).

Intense Pulsed Light:

Intense pulsed light penetrates skin deeper than vascular lasers and targets multiple chromophores including melanin and hemoglobin. Angermeier' study showed that 174 (92.5%) of a total of 188 patients had at least a 75% clearance of their vascular lesions (130). Improvement can be long-term as shown by Weiss and Beasley in a 4 year follow up study (131).

10.5 - Treatment of Phymatous Rosacea:

Isotretinoin been reported to reduce size of the glandular rhinophyma in its early stages, especially if it is accompanied with inflammatory pauples and pustules or oily greasy seborrhoeic skin (132). Pulsed Dye Laser is another form of treatment for the angiomatous type of rhinophyma acting by obliteration of prominent vessels, and the CO2 Laser is effective treatment of rhinophyma where the large distorting nodules can be successfully destroyed with good cosmetic results (133).

There are many more traditional surgical procedures that may also be effective for the nodular lesions in advanced glandular rhinophyma. These include simple shave excision and razor modelling with subsequent healing by granulation and reepithelization. Excision and skin grafting, dermabrassion, cryotherapy and radio-therapy can also have a role (1).

10.6 - Treatment of Ocular Rosacea:

The following are the options for treatment of ocular rosacea (134):

1. Artificial tears frequently needed for dry eyes.
2. Lid and lash hygiene using warm soaks or compressor.
3. Topical antibiotics for infected blepharitis including metronidazole, erythromycin, and fucidic acid.
4. Expression of the meibomian glands by manual massage.
5. Systemic antibiotics including minocycline, doxycycline, oxytetracycline, and erythromycin.
6. Surgery – incision and drainage of the chalazion.

10.7 - New Treatments:

New therapies are being investigated for treatment of rosacea; all are primarily targeted toward control of DF and these include: topical 5% permethrin, topical 1% ivermectin or combination of topical permetherin and oral ivermectin.

Permethrin:

Several case reports showed benefit of use of topical permethrin in rosacea in combination with or after oral ivermectin therapy (135). Aquilina et al, found topical permethrin with single dose of oral invermectin effectively resolve rosacea symptoms in immune compromised patients after failure of topical ketoconazole or metronidazole treatment (136).

Ivermectin:

Ivermectin is a strong acaricide; its efficacy against Demodex was reported in many case reports (137). The significant impact on DF and possibility of anti-inflammatory properties of ivermectin has prompted investigation into development of a topical product for treatment of rosacea. Two randomized, double blind-blind, controlled studies examined the effect of ivermectin 1% cream once daily for 12 weeks in treatment of moderate to severe PPR. The results showed statistically significant improvement of inflammatory lesions in the treatment groups compared to placebo groups ($p<0.001$) (138). As single oral dose of ivermectin has been reported to be effective treatment of a child with ocular and cutaneous rosacea with high density of DF mites which did not respond to doxycyline and isotretinoin treatments (139).

The above studies may confirm the role of DF in the pathogenesis of rosacea particularly PPR and proves that eradication of DF may improve inflammatory lesions of rosacea. However, the number of these studies and patients involved were small and these new treatments need further large studies to prove their efficacy.

11. ANA Blood Test

Serum ANA are defined as all the antibodies that can be detected by indirect immunofluorescence (IIF) and are reactive against nuclei or sometimes to soluble cytoplasmic antigens found in human cells. The current version of the ANA assay used now in most laboratories, including those supplying results for this thesis, employs a human tumour cell line such as Hep-2 for the nucleated cell substrate. The determination of the ANA is made more relevant by a quantitative assay titre, which reflects the serial serum dilutions necessary for fluorescence to disappear. The majority of laboratories set a minimum level of dilution to separate relevant positive tests from non-specific positives with no clinical relevance. This is usually set at 1:80, and this is the level accepted as relevant in this study. It is an effective blood test for screening patients for the presence of CTD including lupus erythematosus. However, positivity of ANA can also occur in patients without underlying autoimmune diseases. Positive insignificant low titre ANA is reported more frequently in females than males and their incidence increases with age, with around a fifth of 60 year old women being non-specifically positive (140).

In one report, based on 15 international laboratories, the ANA positivity rate in the healthy normal populations aged between 20 to 60 years was 13.3% at 1:80, 5% at 1:160 and 3.3% at 1:320 (141). Shu et al, reported an insignificant positive low titre of ANA at 1:10 occurs in 45% of healthy adults between age of 18 and 66 years, a titre of 1:40 occurs in 19%, and titre of 1:80 or more present in 5.6% of the same age group of those healthy adults (142). Anderson et al, found that the incidence of positive ANA of normal adults with age range from 21 years to 40 years was 2%, this incidence increased to 9% of adults with age range from 41 years to 60 years and further increased to 25% of adults over 65 years of age (143), although this seems very high for a UK population, even at low titres. In one Japanese study, investigating

the prevalence of ANA positivity in a general population of 2181 residents of small town, the results showed that 26 % were ANA positive at 1:40 titre and 9.5% were positive at 1:160 dilutions, females having a significantly higher positivity rate than males ($P<0.0001$) (144). Another study from Brazil, of a healthy control population, investigating the ANA positivity of 500 normal individuals, showed that ANA positivity was almost twice as prevalent in females as in males (145).

So, although, ANA is still one of the most important screening and diagnostic tests of CTD however, false positive result of low insignificant titre can be detected in normal populations as in the above studies and this is especially reported more in elderly females. The positive results can be variable between different nations and different laboratory methods used.

In this study both centers (Leeds and Doncaster Hospitals) used the same IIF method and the same substrate (human tumour cell line, Hep-2 cell) and only considered ANA positive if the titre is ≥ 1:80.

Chapter Two – The Study

1. **Aims of the Study**

2. **Method of the Study**

3. **Statistical Analysis**

1. Aims of the study

There is a limited literature examining rosaceous skin disease and its associations particularly with the positivity of ANA and systemic symptoms, within dermatology, and also in rheumatology. Given the frequency of the disease, the possibility of misdiagnosis of rosacea mostly with skin lupus, and the therapeutic implications of this error, the subject is worthy of more significant investigation.

This was principally an observational study, attempting to identify the relationship, if any, between rosacea and immunological and systemic symptomatology suggestive of CTD. The study investigates the frequency of a positive ANA with or without systemic symptoms including myalgia, arthralgia, and Raynaud's phenomenon. It also investigated if there are any associations between rosacea and CTD particularly cutaneous lupus.

This study investigated a large group of patients with rosacea, identified from the Dermatology and Rheumatology Departments in Doncaster and Leeds. It concentrated on clinical elements of the condition, and their relationship to laboratory abnormalities, associated more general symptoms and related CTD.

Patients with more general symptoms were compared with those with only localized skin disease affecting the face in a descriptive analysis intended to aid early detection of those likely to have more generalized symptoms, and also to help early and more specific diagnosis in relation to those disorders often confused with rosacea.

The three principle questions of the study were:

1. **In the cohorts of patients studied, what was the frequency of ANA positivity?**

2. **Is there a sub-group of patients with rosacea that has systemic symptoms with or without immunological abnormalities?**

3. **Is there any relation between rosacea and its sub-types and connective tissue diseases, particularly lupus erythematosus?**

2. Methods

Patients with Rosacea were identified from routine outpatient clinics in Leeds (based at Leeds General Infirmary and Chapel Allerton Hospitals) and Doncaster (based at Doncaster Royal Infirmary and Bassetlaw Hospitals). The principal investigator identified participants from patients attending routine outpatients appointments at the hospital department involved. The study was discussed with potential participants verbally and they were supplied with a written information leaflet (see later) about the study. Subsequently they gave written consent (see later) if they were willing to be involved. Patients who were not able to give the consent for any reason were not included in this study. Patients who did not understand or speak English were only included if they had a family member or interpreter who was able to convey full understanding of the study information sheet.

All information about rosacea was recorded in the rosacea data proforma (see later). This included patient age, sex, duration of rosacea, history of acne vulgaris, family history of rosacea, triggering factors and previous treatment. The clinical pattern of the rosacea was identified (erythemato-telangiectatic, papulo-pustular, phymatous or occular) as well as the skin type. Additional clinical information was documented, concentrating particularly on features suggesting 'systemic' upset, such as arthralgia, myalgia and Raynaud's phenomenon. Previous blood test results were used to identify the presence of ANA with or without the available skin biopsy results for histology and DIF study were all reported.

It was intended that 150 patients should be available for investigation, however I managed to in investigate 169 patients from both Leeds and Doncaster hospitals (93 patients from Doncaster and 76 patients from Leeds) of both sex (male = 77 and female = 92) of different age group (average = 50 years) and different skin types. All patients had ANA blood test recorded but not all patients investigated for the histology or DIF study.

2.1 - Inclusion criteria:

- Rosacea patients who were diagnosed clinically retrospectively in the dermatology departments of Leeds and Doncaster hospitals with or without confirmation of skin histology or DIF test but with an available ANA blood test performed for screening purpose.
- Male and female
- Age 18 and older
- Attending hospital clinics
- Able to give informed consent

2.2 - Exclusion criteria:

- Unable to give informed consent.
- Rosacea patients who had no previous ANA blood test.

2.3 - Control group:

A control group of patients attending a skin cancer screening clinic with no known musculoskeletal diagnosis were asked to be involved, and were asked about the presence of the symptoms described in the questionnaire used for the study group. This was to allow some comparison of features such as arthralgia, myalgia and Raynaud's phenomenon with a local control group, and also to establish consistency of the results with the published literature.

2.4 - Data storage:

Data was stored on hospital-based computers in secure offices. All completed paper recorded data is kept in a locked filing cabinet in a locked office within a security protected NHS hospital dermatology department. Data for analysis did not include any identifiable personal data, but was grouped for disease and results status.

2.5 - Indirect Immunofluorescence (IIF) for the detection of ANA:

This is the method used to detect the ANA in all patients in the study. Both centres used multispot slides containing Hep-2 cells as a substrate to detect the antibodies in a patient's serum. The steps of performing the IIF method are:

Step 1 - Addition of controls and samples:

1. 1 drop of positive control and 1 drop of negative control sera are dispensed to the appropriate slide wells. 20 - 25 µl of fresh diluted patient serum is added to the remaining wells.
2. The slides are placed in a staining container and incubated for 30 minutes. During this incubation period, any anti-nuclear antibodies in the patient's serum will bind to antigens expressed by the Hep-2 cells that are fixed onto each well.
3. After the incubation period, the serum is washed off with buffer the slide is placed into a coplin jar containing wash buffer for approximately 5 minutes.

Step 2 - Addition of fluorescent conjugate:

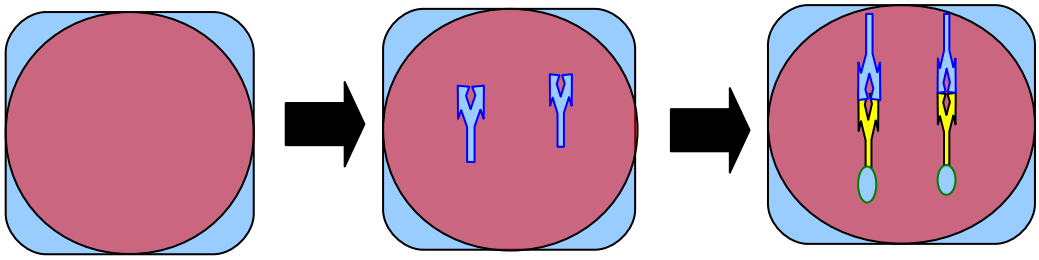
1. Excess wash buffer is removed and 1 drop of fluorescent conjugate (Anti-human IgG) added to each well.
2. The slides are incubated for 30 minutes in a humidified container. During this incubation period, the conjugate will bind to any anti-nuclear antibodies that have bound to Hep-2 cell antigens. This conjugate binding results in the presence of fluorescence in the wells.
3. The slides are re-washed and any excess wash buffer removed.

Step 3 - Identification of positive results:

1. The prepared slides are viewed with a fluorescent microscope, first by scanning 20 or 25x objective to assess cell distribution and uniformity of fluorescence, then with 40x objective to make the final interpretation regarding positivity and pattern.
2. Any fluorescence at ≥ 1:80 dilution is accepted as a positive result.

- Automated Fluorescence Microscopy may be used in modern laboratories.

Figure 21 - IIF steps for ANA test:



(Step 1) **(Step 2)** **(Step 3)**

Figure 21 shows the stages of IIF for the detection of ANA. HEp-2 cells are permeablised (step 1) and then incubated with the patient's blood serum (step 2). If the serum contains antibodies, they will bind to antigens within the HEp-2 cell nucleus. These antibodies can be visualised by subsequent incubation with anti-human antibodies conjugated to a fluorescent molecule (step 3).

2.6 - Rosacea Study Data Proforma:

1. Patient No **2. Sex** **3. Age - (D.O.B)**

4. Skin Type: According to Fitzpatrick skin types (1 – 6).

5. Duration of Rosacea Symptoms: From the start of the first rosacea symptoms.

6. History of Acne Vulgaris: Patients considered having history of acne only, if they have moderate to severe acne and received any form of systemic treatment including different oral antibiotics, retinoids or hormonal treatment either from their general practitioners or by dermatologists.

7. Family History of Rosacea: First degree relatives.

8. History of Steroid Treatment:

- Topical - Any topical steroids from mild to very potent steroids used on the face or neck before rosacea symptoms.
- Systemic - Including oral, intravenous or intramuscular steroids taken before the start of rosacea symptoms. For the intravenous and intramuscular steroids, this means a course of injections and not a single injection.
- Inhalers - Any steroid inhalers either for asthma, allergic rhinitis or others.

9. Triggering Factors:

- Sun
- Cold / Hot weather
- Alcohol
- Spicy Food
- Hot drinks
- Medications
- Others - Include exercise, pregnancy, female period or emotional stress.

10. Associated Systemic Symptoms:

- Flushing - Recurrent flushing not associated with other systemic symptoms that indicate others diseases.
- Eye Symptoms - Any eye symptoms not related to any other eye diseases. Those patients seen previously by ophthalmologists and other eye problems were excluded.
- Myalgia - Muscle pain where the patients need to take pain killers to relieve it and whether it's related or not related to other systemic diseases.
- Arthralgia - Joint pain where the patients need to take regular pain killers and whether it's related to or not related to connective tissue diseases.
- Reynaud's phenomenon - Typical history and symptoms of Raynaud's phenomenon and patients tried some form of treatment either by their general practitioners, dermatologists or rheumatologists.

11. History of Connective Tissue Diseases (CTD):

- Any CTD diagnosed and treated by dermatologists or rheumatologists and recorded in patient's hospital notes.

12. Type of Rosacea:

- Erythemato-telangiectatic Rosacea (ETR) - Erythema, telangiectasia, with or without flushing
- Papulo-pustular Rosacea (PPR) - Papules, pustules, with or without erythema and flushing.
- Phymatous Rosacea (PR) - Mainly Rhinophyma
- Ocular Rosacea (OR) - Diagnosed by ophthalmologists where other eye diseases were excluded.
- Mixed pattern - Any mixed pattern.

13. Investigations:

- Blood - (ANA) - Titre ≥ 1:80 detected by IIF method and using Hep-2 substrate is considered positive in both Leeds and Doncaster hospitals laboratories. All patients were tested for ANA retrospectively.
- Histology - Typical histology features of rosacea. Not all patients had the histology study.
- Direct Immunofluorescence Study (DIF) - Only few patients were tested for DIF.

14. Treatments:

- Topical - Any topical treatment either by general practitioners or dermatologists.
- Oral Antibiotics - Any course of oral antibiotics either by general practitioners or dermatologists. It was difficult to record specific name of antibiotics as many patients tried many courses by their general practitioners before referral to hospital which are not recorded in their hospital notes and was not clearly and specifically mentioned in their general practitioners referral letters.
- Oral Isotretinoin - Always given by dermatologists and recorded in the hospital notes
- Combined Treatments - Any combined treatments of different topical and different systemic.

2.7 - Rosacea Study Participant Information Sheet:

Introduction:

You have been invited to take part in research looking at the features of your skin condition, Rosacea. Please take your time to decide whether you would like to take part and discuss it with your friends and family if you wish. Please ask the study doctor to explain anything you do not understand. Your care will not be affected if you decide not to take part.

You have a skin disease called Rosacea, which is a common skin condition that is estimated to affect about 1 in 10 people in UK and over 45 million people worldwide. Whilst most patients have a rash on their face, with redness, others may get other features such as dilated blood vessels, pus filled spots, gritty eyes, and burning and stinging sensations. Some other patients develop symptoms such as joint aches that may or may not be related to the disease. In these patients, the diagnosis can be unclear, and there may be confusion with other skin diseases. The cause of the disease is unknown, although there are many unproven theories, and the relationship of the various symptoms to the condition may help us to rule out some of these, and develop other more promising ones. There are also a number of methods of treatment for the condition, although we are not studying that aspect in this research.

What is the purpose of this Study?

This study will investigate a large group of patients with Rosacea, and is being carried out to be submitted for a higher medical qualification (an MD or Doctorate of Medicine). It involves collecting information about the way the condition affects you. We will be able to collect most of the information from your hospital records, for instance, the results of laboratory tests, but may need to talk to you about the

symptoms that the condition causes. This will allow us to compare the condition in a large number of patients, and allow us to detect links to other diseases.

Why have I been chosen?

You are being asked to take part in this research because you are known to have Rosacea.

Do I have to take part?

No, your participation is voluntary and your care and treatment will not be affected by any decision you make about taking part in the study. If you wish to withdraw from the study, you are free to do so at any time and this will not affect your future treatment.

What do I have to do?

In order to participate in the study we will ask you to sign a consent form and the study doctor will ask you few questions about your condition. Then, we will collect some details, including the results of previous tests such as skin biopsy and blood results from your hospital notes. We will not be performing any additional tests that have not already been performed by the doctor who normally looks after your skin problem.

What are the possible disadvantages and risks of taking part?

There are no risks at all. You only need to attend an outpatient appointment where you been treated before for about 20 minutes only once.

What are the benefits of taking part?

There are no personal benefits for you; however, this study will extend our understanding of Rosacea.

Will my taking part in the study be kept confidential?

All the information given for the purpose of this study will be treated confidentially.

What will happen to the results of the research study?

When the study has been completed, we will aim to publish the results in a peer-reviewed journal. Please let us know if you wish to be informed of the publication of the study and we will aim to keep you fully informed. You will not be identified in any publication.

Will you inform my GP Doctor?

Yes, we will inform your GP doctor about your participation in this study unless you advise us not to inform them, we will also keep your doctors informed about your future skin management as before

I am interested in taking part in this study – What do I do now?

Contact the Research Team directly as below and we will give you an appointment to come to Dermatology Outpatients Department either in Leeds General Infirmary Hospital or Doncaster Royal Infirmary Hospital depends on your convenience.

Whom can I contact for participation in this study or for any further information?

Dr. Mark Goodfield - 0113 3922581 (Leeds General Infirmary Hospital)

Dr. Mustafa Marai - 01302 366666 (Doncaster Royal Infirmary Hospital)

2.8 - Consent Form:

Title of Project: A clinical and Pathological Investigation of Rosacea.

Name of Researcher: Dr. Mustafa Marai

Title: **Initial:** **surname:**

1. I confirm that I have read and understand the information sheet dated **22/04/2009** - **Version (3**) for the above study. ☐

2. I have had the opportunity to consider the information, ask questions and have had these answered satisfactory. ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without my medical care or legal rights being affected. ☐

4. I understand that relevant sections of my medical notes and data Collected during the study may be looked at by individuals, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

5. I understand that my details will be recorded in the Proforma Sheet (Study questionnaire interview sheet), so it will be identifiable and will be stored on hospital-based computers. All completed paper recorded data will be kept in a locked filing cabinet in locked office within security protected NHS Hospital Dermatology Department.

Data for analysis for publication in future will not include any of my Identifiable personal data, ☐

6. I agree that skin biopsies and blood tests already taken may be used for the research. ☐

7. I agree to my GP being informed of my participation in the study. ☐

8. I agree to take part in the above study. ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Person Taking consent	Date	Signature

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes.

3. Statistical Analysis

3.1 - Sample Size Calculations:

Whilst the majority of the analyses in this retrospective observational study are descriptive, multivariate binary logistic regression was used to identify the features that are most helpful in distinguishing patients with more generalised symptoms. I anticipated that up to 6 of the features under investigation may prove to be independently associated with symptom patterning in a multivariate model, therefore; I amid to recruit a minimum of 150 patients.

3.2 - Statistical Analysis:

Descriptive statistics were used to compare the characteristics of the two groups of patients with rosacea, and features associated with ANF positivity were identified. Descriptive summary statistics provided are mean and N (%) for categorical variables. I used two-sided tests throughout my statistical analysis and the $p < 0.05$ as my criterion for statistical significance. The statistical tests I used in the analysis of my data include:

1. Chi-Squared Test / Test of Association (Dependence):

A chi-square test is used to check the relationship between two categorical variables. It gives an estimate on the agreement between a set of observed data and a random set of data that I expected the measurements to fit. The calculated chi squared correlated to p-value. I used this test to check the relationships between:

- Subtypes of rosacea, and history of acne vulgaris, (Table 1.7).
- Subtypes of rosacea and family history of rosacea, (Table 1.9).
- Antinuclear antibody (ANA) and systemic symptoms, (Table 2.7).

2. A one Sample Binomial Test:

The one sample binomial test used to check whether the proportion of successes on two level categorical dependent variable significantly differs from the hypothesized value. The binomial test is an exact test of the statistical significance of deviations from a theoretically expected distribution of observations into two categories. I used this test to show whether the proportion of patients with or without connective tissue disease (CTD) who were ANA positive different from the proportion observed in the general population in both Leeds and Doncaster groups, (Table 2.5).

3. Fisher's Exact Test:

The Fisher's exact test is used when the chi-square test cannot be conducted because one or more of the cells have an expected frequency of five or less. In the case of analyzing marginal conditions, the p value can be found by summing the Fisher's exact values for the current marginal configuration and each more extreme case using the same marginal's. I used this test to check the effect of oral antibiotics on the positivity of ANA in presence or absence of connective tissue disease (CTD), (Table 2.9).

4. Cochran’s Test / Test of Conditional Association (Dependence):

This test is used to extend the chi-square test of independence in a 2 X 2 table to multiple 2 X 2 tables where each table corresponds to a different level of an intervening variable. This test has conditional independence-independence of the variables forming the rows and columns of the table, conditional on the levels of a third variable. I used this test to check:

- The relationship of positivity of ANA and different rosacea subtypes conditioned by presence or absence CTD (Table 2.6).
- The relationship of systemic symptoms and different rosacea subtypes conditioned by positive or negative ANA result (Table 2.8).

5. Breslow-Day Test for Homogeneity of the Odds Ratio:

This test statistic sums the squared deviations of observed and fitted values each standardized by its variance. The test is used for stratified analysis of 2x2 tables to test null hypothesis that the odds ratios for the strata are all equal. When the null hypothesis is true, the statistic has an asymptotic chi-square distribution. I used this test to check the homogeneity of the odds ratio, (Table 2.6) and (Table 2.8).

6. Binary Logistic regression:

It is a type of probabilistic statistical classification model. It is used to predict a binary response from a binary predictor, categorical or continuous predictor variables. It is used for predicting the outcome of a categorical dependent variable based on one or more predictor variables (features). I used this test to check if any of the rosacea subtypes were associated with ANA positivity having controlled centre (Doncaster and Leeds), CTD, and oral antibiotic treatment, (Table 2.10).

Chapter Three – Results

1. **Analysis of Dermographic Data**

2. **Analysis of patients with Positive ANA**

1. Demographic Data of Doncaster and Leeds patients

The results are shown in a series of tables below (Table 1.1 to Table 1.15).

Table 1.1 - Gender:

Gender	Doncaster (93 patients)	Leeds (76 patients)	Doncaster + Leeds (169 patients)
Male	42 (45%)	35 (46%)	77 patients (45%)
Female	51 (55%)	41 (54%)	92 patients (55%)

Table 1.2 - Age:

Age	Doncaster (93 patients)	Leeds (76 patients)
Male	33 – 81 Years Average 58 years	29 – 90 Years Average 53 years
Female	26 – 79 Years Average 50 years	31 – 70 Years Average 49 years
Overall Range	53 Years	50 Years

Tables 1.1 and 1.2 show the basic demographics, indicating broad comparability between the age and sex distributions of the two groups derived from Doncaster and Leeds. The Leeds group is marginally younger on average, but this difference is not significant.

Table 1.3 - Skin type:

Skin Type	Doncaster (93 patients)	Leeds (76 patients)	Doncaster + Leeds (169 patients)
Type 1	86 patients (92%)	14 patients (18%)	100 patients (59%)
Type 2	7 patients (8%)	29 patients (38)	36 patients (21%)
Type 3	0 (0%)	28 patients (37%)	28 patients (17%)
Type 4	0 (0%)	0 (0%)	0 (0%)
Type 5	0 (0%)	4 patients (5%)	4 patients (2%)
Type 6	0 (0%)	1 patient (1%)	1 patient (1%)

With regard to skin type (Table 1.3), the patients from Doncaster are much more typical of the stereotype for patients with rosacea, being of overwhelmingly skin type 1. In contrast, Leeds patients were of a wider range of skin types, including 5% of patients with skin type 5. This suggests that there may be different aetiological factors at work in the Leeds group, and the further data suggest some potential explanations to be discussed later.

Table 1.4 - Duration of rosacea symptoms:

Doncaster (93 patients)	Leeds (76 patients)
12 - 564 months Average = 87 months	1- 500 months Average = 54 months

There was some difference in the duration of the disease between the two groups (Table 1.4), with the mean in Doncaster being 87 months whilst that for Leeds was 54 months, and this was significant statistically. There was little difference in the range of disease duration.

Table 1.5 - Rosacea subtypes:

Rosacea Subtype	Doncaster (93 patients)	Leeds (76 patients)	Doncaster + Leeds (169 patients)
Prevalence of different rosacea subtypes			
ETR	63 (68%)	47 (62%)	110 (65%)
PPR	56 (60%)	72 (95%)	128 (76%)
PR	17 (18%)	8 (11%)	25 (15%)
OR	5 (5%)	5 (7%)	10 (6%)
Isolated rosacea subtypes patten			
ETR	27 (29%)	4 (5%)	31 (18%)
PPR	20 (22%)	27 (36%)	47 (28%)
PR	4 (4%)	0 (0%)	4 (2%)
Total isolated	51 (55%)	31 (41%)	82 (49%)
Mixed rosacea subtypes pattern			
ETR + PPR	26 (28%)	32 (42%)	58 (34%)
ETR + PR	4 (4%)	0 (0%)	4 (2%)
PPR + PR	5 (5%)	2 (3%)	7 (4%)
PR + OR	1 (1%)	0 (0%)	1 (1%)
ETR + PPR + PR	2 (2%)	6 (8%)	8 (5%)
ETR + PPR + OR	3 (3%)	5 (7%)	8 (5%)
ETR + PR + OR	1 (1%)	0 (0%)	1 (1%)
Total mixed	42 (45%)	45 (59%)	87 (51%)

Table 1.5 shows the prevalence of rosacea subtypes where the PPR (128 patients - 76%) and ETR (110 patients - 65%) were the commonest presenting rosacea subtypes. The most combined rosacea subtypes were ETR and PPR (58 patients - 34%) more than any other mixed pattern. Half of recruited patients 51% had mixed rosacea sub-types, while only 2% had isolated PR, however the OR was only present in those with skin manifestations, and never occurring alone. Whilst 29% of Doncaster patients had ETR alone, the figure for Leeds patients was only 5%. In contrast, 36% of Leeds patients had isolated PPR disease, compared to 22% in the Doncaster cohort. This difference may be consistent with the frequency of Type1 skin

in Doncaster patients, since ETR is common in those with Celtic skin. The corollary is that the Leeds patients with a broader skin-type mix might be more prone to papulo-pustular disease as a predominant sub-type.

Table 1.6 - History of acne vulgaris:

Doncaster (93 patients)	Leeds (76 patients)	Doncaster + Leeds (169 patients)
30 patients (32%)	29 patients (38%)	59 patients (35%)

Table 1.6 shows that a history of acne vulgaris was described by 35% of patients overall, with no significant difference between the two groups.

Table 1.7 - Association between history of acne and rosacea subtypes:

Acne	Rosacea Subtypes - No, % present			
	ETR	PPR	PR	OR
Absent	74/110 67.3%	82/110 74.5%	9/110 8.2%	8/110 7.3%
Present	36/59 61.0%	46/59 78.0%	16/59 27.1%	2/59 3.4%
Pearson's chi-square	X^2=0.02 p=0.878	X^2=0.25 p=0.621	X^2=10.93 p=0.001	X^2=1.04 p=0.308

Table 1.7 shows the relationship between a history of acne and rosacea subtypes. The history of acne vulgaris in rosacea was significant among PR subtypes (p=0.001). The numbers are small, but this is an interesting observation that may deserve further investigation.

Table 1.8 - Family history of rosacea:

Doncaster (93 patients)	Leeds (76 patients)	Doncaster + Leeds (169 patients)
16 patients (17%)	8 patients (11%)	24 patients (14%)

Table 1.8 shows 14% of patients had a family history of the rosacea (Doncaster 17% and Leeds 11%) the difference is not significant.

Table 1.9 - Association between family history of rosacea and rosacea subtypes:

Family History	Rosacea Subtypes - No, % present			
	ETR	**PPR**	**PR**	**OR**
Absent	90/145 62.1%	111/145 76.6%	19/145 13.1%	8/145 5.5%
Present	20/24 83.3%	17/24 70.8%	6/24 25.0%	2/24 8.3%
Pearson's chi-square	X^2=4.10 p=0.043	X^2=0.37 p=0.545	X^2=2.31 p=0.128	X^2=0.29 p=0.588

Table 1.9 shows the positive family history of rosacea in the participated patients was not highly significantly different between subtypes of rosacea (24 out of total 169 patients - 14%), and the most positive family history of rosacea was seen in the ETR subtype (20 out of total 24 patients - 83%), and although the numbers are small, this would be consistent with the known links with Type 1, Celtic skin types.

Table 1.10 - History of previous steroid treatment:

Steroid Treatment	Doncaster (93 patients)	Leeds (76 patients)	Doncaster + Leeds (169 patients)
Topical	17 patients (18%)	41 patients (54%)	58 patients (34%)
Systemic	10 patients (11%)	21 patients (28%)	31 patients (18%)
Inhalers	10 patients (11%)	5 patients (7%)	15 patients (9%)

Table 1.10 shows the history of previous steroid treatment in two groups of rosacea. The topical treatment was reported by 34% of patients, with a significant difference between Leeds (54%) and Doncaster (18%). Also the use of systemic steroid treatment was more in Leeds compared to Doncaster groups (28% ves. 11%). This may have direct relevance both to the sub-types of disease, with papulo-pustular disease common in Leeds where steroid use was commonly reported, and ETR more common in Doncaster, where steroid use was less common and skin type 1 patients predominated.

Table 1.11 - Associated systemic symptoms:

Associated Symptoms	Doncaster (93 patients)	Leeds (76 patients)	Doncaster + Leeds (169 patients)
Flushing	53 patients (57%)	64 patients (84%)	117 patients (69%)
Eye Symptoms	34 patients (37%)	8 patients (11%)	42 patients (25%)
Arthralgia	21 patients (22%)	15 patients (20%)	36 patients (21%)
Reynaud's	16 patients (17%)	12 patients (16%)	28 patients (17%)
Myalgia	18 patients (19%)	5 patients (7%)	23 patients (14%)

Many patients reported a number of associated systemic symptoms (Table 1.11). Surprisingly, given the nature of the clinics from which the subjects were drawn in the two centers, joint symptoms, Raynaud's phenomenon and muscle aches were equally common in both cohorts. Around 15 – 20% of patients had one or other of these symptoms. The arthralgia and myalgia had the same percentage as reported by the control group (Table 1.12) and normal populations; however the Raynaud's phenomenon was reported in about 16% in both groups which is greater than reported in the control group and the general populations. This is could be an inverse relationship where the treatment of Raynaud's phenomenon by the calcium channel blocker (vasodilators) triggers the flushing symptom of rosacea. Facial flushing and eye symptoms were common in both groups. Flushing occurred in 84% of patients in Leeds, even though PPR was more common here. A quarter of patients had eye symptoms, although it is not clear that all of these were due to rosaceous eye disease.

Table 1.12 - Systemic symptoms frequency in control group:

Symptoms	**Number (%)**
Flushing	5 patients (8.3 %)
Eye Symptoms	7 patients (12%)
Arthralgia	11 patients (18.5%)
Raynaud's	2 patients (3.5%)
Myalgia	8 patients (13.3%)

Table 1.12 shows the frequency of comparable symptoms in the control group of non-rosacea patients of 60 controls (35 female and 25 male) with mean age of 43 years (age range 17 – 92 years).

Table 1.13 - Triggering factors:

Triggering Factor	Doncaster (93 patients)	Leeds (76 patients)	Doncaster + Leeds (169 patients)
Sun	46 patients (49%)	46 patients (61%)	92 patients (54%)
Alcohol	33 patients (35%)	54 patients (71%)	87 patients (51%)
Cold / Hot Weather	71 patients (76%)	9 patients (12%)	80 patients (47%)
Spicy Food	24 patients (26%)	51 patients (67%)	75 patients (44%)
Hot Drinks	9 patients (10%)	16 patients (21%)	25 patients (15%)
Stress	9 patients (10%)	4 patients (5%)	13 patients (8%)
Medications	2 patients (2%)	8 patients (10%)	10 patients (6%)
Exercise	3 patients (3%)	0 (0%)	3 patients (2%)
Female Periods	2 patients (2%)	0 (0%)	2 patients (1%)
Pregnancy	1 patient (1%)	1 patient (1%)	2 patients (1%)

Table 1.13 shows the variations seen in triggering factors reported of rosacea. Sun was a factor in both cohorts, but temperature change as a triggering factor was much more frequently reported in Doncaster (76% of patients), whilst only 12% of patients reported in Leeds. This suggests that temperature change may be more relevant to ETR than to PPR. However, spicy food was more commonly a problem in Leeds, reported in 67% of subjects, but only in 26% of patients in Doncaster. The same was true for alcohol (71% of patients in Leeds, but only 35% of patients in Doncaster). The remainder of the reported precipitating factors occurred in only small numbers of patients and were not statistically different between the two groups. Overall, only 6% of patients documented that medication had affected their disease, although this figure was 10% in Leeds. The results do not tell us whether this related to steroid treatments or other medications.

Table 1.14 - Investigations:

Investigation	Doncaster (93 patients)	Leeds (76 patients)	Doncaster + Leeds (169 patients)
Blood - Positive ANA	6 Patients (6%)	16 patients (21%)	22 patients (13%)
Histology – Rosacea Features	10 patients (11%)	23 patients (30%)	33 patients (20%)
DIF – Negative	8 patients (9%)	6 patients (8%)	14 patients (8%)
DIF – Positive	0 (0%)	1 patient (1%)	1 patient (0.5%)

Not all patients had a diagnosis confirmed by histology, since in many the diagnosis was clinically apparent in Table 1.14. Only 20% of patients were biopsied overall, with more in Leeds (30%), compared to Doncaster (11%). This represents the differing clinical settings in which these patients were seen. The biopsy for histology and DIF is much more likely when there is a specific need to exclude lupus as the cause of the facial rash, particularly in those with papular disease occurring without pustules, and occasionally in patients who were already known to have lupus. In those who did have a biopsy showing histological features of rosacea, and had immuno-fluorescent studies performed, a positive test was seen in only one. On the other hand, ANA positivity occurred in 21% of patients from Leeds, but in only 6 patients (6%) of those in Doncaster.

Table 1.15 - Treatments:

Treatment	Doncaster (93 patients)	Leeds (76 patients)	Doncaster + Leeds (169 patients)
Topical	86 patients (93%)	67 patients (88%)	163 patients (96%)
Oral Antibiotics	78 patients (84%)	61 patients (80%)	139 patients (82%)
Topical + Oral Antibiotics	73 patients (78%)	56 patients (73%)	129 patients (76%)
Oral Isotretinoin	19 patients (20%)	14 patients (18 %)	33 patients (19%)
Oral Antibiotics and Isotrtinoin	18 patients (18%)	11 patients (14.5%)	29 patients (17%)

Treatment of rosacea was remarkably consistent (Table 1.15) between the two cohorts, and was consistent with modern practice and NICE guidance. Most patients received different topical acne treatment (96%) and one or more of oral antibiotic courses (82%). Approximately 20% of patients received oral isotretinoin.

2. Doncaster and Leeds Patients with Positive ANA Blood Test

Tables 2.1 to 2.10 present the demographic data on these patients, and the analysis compares the characteristics of those with and without a positive ANA.

There are significant differences in both age and gender distribution, with ANA positive patients being predominantly female (indeed entirely female in Doncaster), and markedly younger than the overall group. This is consistent with the well documented relationships between positive ANA status and young females (Table 2.1 and 2.2).

Table 2.1 - Number of patients with positive ANA:

Centre, Total patients	**No, % of ANA positive patients**
Doncaster (93 Patients)	6 patients (6%)
Leeds (76 Patients)	16 patients (21%)
Doncaster + Leeds (169 patients)	22 patients (13%)

Table 2.2 - Age and gender of patients with positive ANA:

Patients with Positive ANA	**Gender**	**Age**
Doncaster (6 patients)	6 Female (100%)	36 – 79 years (Average 49 Years)
Leeds (16 patients)	13 Female (81%) 3 Male (19%)	31 – 68 Years (Average 45 years) 31 – 45 Years (Average 38 Years)
Doncaster + Leeds (22 Patients)	19 Female (86%) 3 Male (14%)	31 – 79 Years (Average 47 Years) 31 – 45 Years (Average 38 Years) Overall Average 46 Years

Table 2.3 - Type of rosacea in patients with positive ANA:

Type of Rosacea	Doncaster (6 patients)	Leeds (16 patients)	Doncaster + Leeds (22 patients)
ETR	3 patients (50%)	1 patient (6%)	4 patients (18%)
PPR	1 patients (17%)	7 patients (44%)	8 patients (36%)
ETR + PPR	6 patients (38%)	2 patients (33%)	8 patients (36%)
ETR + PPR + OR	2 patients (33%)	0 (0%)	2 patients (9%)

Table 2.3 shows the types of rosacea associated with a positive ANA, these subtypes were distributed in the same way as the subtypes in the overall groups where the PPR is more in the Leeds group and the ETR is more in the Doncaster group.

Table 2.4 - History of CTD in patients with positive ANA:

Connective Tissue Disease	Doncaster (6 patients)	Leeds (16 patients)	Doncaster + Leeds (22 patients)
Lupus Erythematosus	1 patient (17%)	11 patients (69%)	12 patients (55%)
Rheumatoid Arthritis	0 (0%)	5 patients (31%)	5 patients (23%)
Dermatomyositis	0 (0%)	1 patient (6%)	1 patient (5%)
Scleroderma	0 (0%)	1 patient (6%)	1 patient (5%)

Table 2.4 shows total number of patients with history of CTD and the relationship of positive ANA rosacea patients with history of CTD. Not surprisingly, in the Leeds cohort, the ANA positive patients had a history of CTD in all cases (16 patients) and some patients had more than one CTD. In Doncaster group, only 1 patient (17%) out of 6 patients positive ANA had history of lupus.

Table 2.5 - Prevalence of ANA positivity in patients with or without history of CTD:

CTD	Centre	ANA	
		Negative	Positive
No	Leeds	57 95%	3 5%
	Doncaster	87 94.6%	5 5.4%
	Total	144 94.7%	8 5.3%
Yes	Leeds	3 18.8%	13 81.3%
	Doncaster	0 0.00%	1 100%
	Total	3 17.6%	14 82.4%
Total	Leeds	60 78.9%	16 21.1%
	Doncaster	87 93.5%	6 6.5%
	Total	147 87%	22 13%

Although, on first appearances a greater proportion of Leeds patients were ANA positive (21.1%, 16/76) versus Doncaster patients (6.5%, 6/93), this was due to the fact that more Leeds patients had a history of CTD than Doncaster patients. In patients without a history of CTD, the level of ANA positivity of both centres combined (5.3%, 8/152) was similar to that reported in the general population; (One-sample Binomial test compared to null hypothesis proportion [5%] p=0.500). Patients with a history of CTD, were much more likely to be ANA positive (both centres combined 82.4% (14/17); (One-sample Binomial test compared to null hypothesis proportion [5%] z=14.08, p<0.001).

Table 2.6 - Prevalence of ANA positivity in different rosacea subtypes in presence or absence of CTD:

CTD	Rosacea Subtypes	Rosacea Subtype - No, % ANA positive			
		ETR	PPR	PR	OR
No	Absent	3/53 5.7%	3/40 7.5%	8/127 6.3%	8/144 5.6%
	Present	5/99 5.1%	5/112 4.5%	0/25 00.0%	0/8 00.0%
	Total	8/152 5.3%	8/152 5.3%	8/152 5.3%	8/152 5.3%
Yes	Absent	5/6 83.3%	1/1 100.0%	14/17 82.4%	12/15 80.0%
	Present	9/11 81.8%	13/16 81.3%	n/a*	2/2 100.0%
	Total	14/17 82.4%	14/17 82.4%	14/17 82.4%	14/17 82.4%
Total	Absent	8/59 13.6%	4/41 9.8%	22/144 15.3%	20/159 12.6%
	Present	14/110 12.7%	18/128 14.1%	0/25 00.0%	2/10 20.0%
	Total	22/169 13.0%	22/169 13.0%	22/169 13.0%	22/169 13.0%
Breslow-Day		$X^2 = 0.00$ p = 0.992	$X^2 = 0.13$ p = 0.715	n/a**	$X^2 = 0.97$ p = 0.325
Cochran's		$X^2 = 0.03$ p = 0.859	$X^2 = 0.71$ p = 0.398	$X^2 = 1.66$ p = 0.197	$X^2 = 0.01$ p = 0.932

*None of the patients with a history of CTD had PR.

**Tests of homogeneity of odds ratio could not be performed.

Table 2.6 shows the relationship of positivity of ANA in different rosacea subtypes conditioned by effect of history of CTD. Splitting the analysis by CTD did not indicate that the associations between specific types of rosacea and ANA positivity differed depending on CTD status (Breslow-Day tests of homogeneity of the odds ratio supported this). For example, in absence of CTD, the positivity of ANA in ETR

patients is 5.1% which is nearly the same percentage among non ETR patients (5.7%) and in presence of history of CTD, the percentage of positivity of ANA in ETR is 81.8% which is not hugely different from other non ETR patients (83.3%). The total percentage of ETR patients with positive ANA and with or without history of CTD is 12.7% which is very close when compared to non ETR patients (13.6%). This trend is nearly the same with other rosacea subtypes. So, the descriptive data did not highlight any conspicuous trends i.e. having a particular subtype of rosacea (whether or not a patient also had CTD) did not seem to greatly influence the odds of being ANA positive, and this was supported by Cochran's tests of conditional independence.

Table 2.7 - Association between ANA and systemic symptoms:

ANA	**Systemic Symptoms - No, % Present**			
	Myalgia	**Arthralgia**	**Raynaud's**	**Mixed Symptoms**
Negative	16/147 10.9%	21/147 14.3%	16/147 10.9%	34/147 23.1%
Positive	07/22 31.8%	15/22 68.2%	12/22 54.5%	17/22 77.3%
Total	23/169 13.60%	36/169 21.3%	28/169 16.6%	51/169 30.2%
Pearson's Chi-Square	$X^2 = 7.13$ p= 0.008	$X^2 = 33.10$ p<0.001	$X^2 = 26.39$ p<0.001	$X^2 = 26.62$ p<0.001

Around a third of patients (30.2%) had mixed systemic symptoms; patients who tested positive for ANA were more likely to have systemic symptoms (77.3%) than patients who tested negative for ANA (23.1%, p<0.001). The largest effect was observed for arthralgia (68.2% ves. 14.3%, p<0.001). This appeared to be simply due to the increased frequency of real CTD in those who were ANA positive.

Table 2.8 - Prevalence of systemic symptoms in different rosacea subtypes in positive and negative ANA patients:

Rosacea Subtypes	ANA	Rosacea subtype - No, % with Systemic Symptoms			
		ETR	PPR	PR	OR
Absent	Negative	13/51 25.5%	12/37 32.4%	28/122 23.0%	30/139 21.60%
	Positive	7/8 87.5%	2/4 50.0%	17/22 77.3%	15/20 75.00%
	Total	20/59 33.9%	14/41 34.1%	45/144 31.3%	45/159 28.30%
Present	Negative	21/96 21.9%	22/110 20.0%	6/25 24.0%	4/8 50.00%
	Positive	10/14 71.4%	15/18 83.3%	n/a*	2/2 100.00%
	Total	31/110 28.2%	37/128 28.9%	6/25 24.0%	6/10 60.00%
Breslow- Day		X^2=0.43 p=0.512	X^2=3.67 p=0.057	n/a**	X^2=0.19 p=0.665
Cochran's		X^2 =26.6 p <0.001	X^2=27.3 p<0.001	X^2=25.6 p<0.001	X^2=26.1 p<0.001

*None of the patients with PR were ANA positive.

**Tests of homogeneity of the odds ratio could not be performed.

Table 2.8 shows the prevalence of systemic symptoms in different rosacea subtypes conditioned by effect of ANA result. The results showed that the PPR subtype had systemic symptoms and positive ANA when compared to other non PPR subtypes (83.3% ves. 50.0%). This has some indication that in patients with PPR the association between ANA and systemic symptoms was stronger than it was in patients without PPR (Breslow-Day test of homogeneity of odds ratio p=0.057). However, only a small number of patients without PPR were ANA positive (n=4). Therefore, we cannot conclude that this was a genuine effect. This would need to be confirmed in a larger study.

Table 2.9 - The influence of oral antibiotics on patients with positive ANA:

CTD	Antibiotics	ANA - No, %	
		Negative	Positive
No	No	23 (92%)	2 (8%)
	Yes	121 (95.3%)	6 (4.7%)
	Total	144 (94.7%)	8 (5.3%)
Yes	No	0 (0.00%)	5 (100%)
	Yes	3 (25%)	9 (75%)
	Total	3 (17.6%)	14 (82.4%)
Total	No	23 (76.7%)	7 (23.3%)
	Yes	124 (89.2%)	15 (10.8%)
	Total	147 (87%)	22 (13%)

Patients taking antibiotics were if anything less likely to be ANA positive, whether or not they had a history of CTD; there was no statistically significant effect; combined across CTD status 23.3% of patients not taking antibiotics (7/30) tested positive for ANA compared to 10.8% of those taking antibiotics (15/139); (Fisher's exact test p=0.076). The previous antibiotics therapy did not appear to influence ANA positivity. This is important because tetracycline antibiotics in general and minocycline in particular, are well known to increase antibody positivity.

Table 2.10 - Binary Logistic Regression:

A multiple exact binary logistic regression model was used to assess whether any of the specific types of rosacea were associated with ANA positivity having controlled for centre, CTD and oral antibiotics.

Variable	Odds Ratio (95% CI)	p-value
Centre	1.0 (0.2 - 7.4)	1.000
History of CTD	53.3 (9.9 - 471.6)	<0.001
Antibiotic Treatment	0.5 (0.1 - 2.8)	0.525
ETR	0.6 (0.1 - 3.4)	0.721
PPR	0.4 (0.0 - 3.5)	0.607
PR	0.4 (0.0 - 2.9)	0.414
OR	1.7 (0.1 - 41.2)	1.000

Whilst history of CTD increased the odds of a patient being ANA positive, neither rosacea subtypes nor antibiotic treatment were associated with ANA status.

Chapter Four – Discussion

1. **Demographic Data**
2. **Positive ANA Patients**
3. **Effect of Oral Antibiotics on ANA**
4. **Conclusions**
5. **Strength of the Study**
6. **Limitations of the Study**
7. **Weakness of the Study**
8. **Recommendations**

1. Demographic Data

Rosacea is a common disease that predominantly, but not only, affects middle aged women with fair skin. It most often affects the face, occasionally the neck and chest and rarely other sites. There are 4 major subtypes and these can present at different stages in the same patient at different times or can be consistent throughout a patient's history. There are many differential diagnoses for rosacea, and with regard to this study, the confusion with lupus erythematosus, especially when it resembles the facial erythema of LE, or the papular form of rosacea which is easily confused with rosaceous LE, is very important.

In this thesis, I have investigated the hypothesis that patients with rosacea may have a positive ANA blood test and also that this may be associated with systemic symptoms in a patient who does not have CTD. I have also investigated the possibility that certain CTDs, particularly lupus, may occur concurrently with rosacea. There are very limited data on this subject and very few publications. To my knowledge, this is the largest study in terms of the number of patients in which the prevalence of a positive ANA blood test has been investigated and related to the various subtypes of rosacea. I have also produced data on the presence of CTD in the different subtypes of rosacea.

Most of the 169 patients were diagnosed clinically after a full history and clinical examination. All the associated symptoms, triggering factors or any other history related to rosacea were documented from the clinical interview. All patients had a blood test to detect the presence of ANA and this is considered positive if the titre ≥ 1:80. The test was a pre-requisite to inclusion in the study. And this may have influenced the results, since it is possible that some patients had the blood test performed because of the suspicion of an alternative diagnosis, but the overall data

are consistent with other published studies (140 -145) so this is less likely to be a confounding factor. The overall frequency of ANA positivity was 13%, but when those patients with a known CTD were excluded, the figure was close to that reported in normal populations (i.e. around 5%). The age and sex distribution of the patient group is consistent with that reported in the earlier literature. The skin type of the patients was one of the interesting variables, being significantly different between the two centres. Type 1 skin was much more prevalent in the cohort derived from Doncaster, whilst the Leeds cohort included a significant proportion of patients with types 3, 4 and even 5. This difference may be relevant to other differences between the cohorts and may be a result of the much larger numbers of patients with co-existent CTD seen in Leeds. Other results suggest that treatment exposure, particularly to steroid preparations, may be implicated in the aetiology of the rosacea in some of these patients, and in this circumstance, the role of skin type may be less important. Most of the investigated patients presented with combined rosacea subtypes of ETR and PPR more often than a single subtype 34%. More than half of recruited patients (51%) had mixed rosacea subtypes, with only 2% with isolated PR. However, because of the recruitment methodology, the OR wasn't present alone, but could only be seen in patients who presented with skin manifestations. This may explain the low overall frequency of ocular disease when compared to the published literature.

The data showed that 35% had a history of acne vulgaris (that required systemic treatment either by at least one course of oral antibiotic, oral retinoids or hormonal treatment), which was more significant among PR subtypes (p=0.001). The numbers were small, but this is an interesting observation that may deserve further investigation. There are a very limited number of studies that have examined the rosacea relationship with acne. However, to my knowledge, there were no studies checked the relationship between rosacea subtypes and history of acne. In a survey reported by the NRS, of more than 100 women with rosacea, 40% of these patients

provided a history of acne in adolescence. Furthermore, about 40% of those women who had a history of acne exhibited moderate flushing triggered by the same triggers common to rosacea (146). In a further investigation of rosacea patients compared to controls, researchers found that rosacea patients had nearly twice the rate of facial sebum production, numbers of microcomedones and propionibacterium acnes bacteria compared to controls (146).

The family history was not a significant finding, since only 14% recorded a positive family history of rosacea in first degree relatives. In many surveys performed by the NRS, a strong positive family history in one close family member was often seen (12), (13). There were 34% of patients who recorded the use of topical steroids as treatment for their skin problem at one stage. However, none of these patients were using topical steroids during the time of study and all of them confirmed that they had stopped using these agents some time before participating in the study. Also 18% gave a history of taking systemic steroids in the past before the appearance of rosacea. The role of steroid usage in association with rosacea and steroid induced rosacea has been discussed on multiple occasions in the literature (86 - 89), as well as previously in this thesis. It is particularly interesting that steroid usage was much higher in the Leeds cohort, and that the pattern of rosacea in this group was significantly more PPR than ETR. It seems a reasonable conclusion that this may well be relevant, particularly in those patients thought to have a co-existent CTD since they are likely to have used both local and oral steroid. Steroid usage may well be one of the more important reasons for differences between the cohorts and for the occurrence of rosacea in patients with co-existent CTD derived from the Leeds clinics. With regard to the presence of associated systemic symptoms in patients with rosacea, a number were specifically recorded: a history of flushing, eye symptoms, arthralgia, Raynaud's phenomenon and myalgia symptom were enquired for. Out of these potentially systemic symptoms, not surprisingly, flushing was the most common associated symptom and affected more than two thirds of patients in

both Doncaster and Leeds. This was not associated with any other symptomatology and was almost certainly simply a feature of the rosacea itself. In terms of frequency, this was followed by eye symptoms including itching, burning and soreness, as well as redness which were recorded in up to 25% of Doncaster and Leeds patients. The frequency of eye disease in the reported literature is up to 50%, a figure which is very high compared to the current data, and feels high compared to that found in routine practice. It is interesting, however, that eye disease is reported to occur more frequently in patients with marked flushing, and that does seem to be the case here. Symptoms potentially more related to CTD, including myalgia, Raynaud's phenomenon and arthralgia were present in less than a quarter of patients, between 14% and 21% depending on the symptom. The frequency of occurrence of Raynaud's phenomenon in the general population is between 3 and 5% (147), and that of non-specific arthralgia around 20% (148), so in this data set, Raynaud's is more common than would be expected, and this could be an inverse relationship where the treatment of Raynaud's with vaso-dilators such as calcium channel blockers might trigger flushing symptom in rosacea patients. The arthralgia and myalgia occurred in the anticipated frequency. To my knowledge there have been no previous studies that have investigated the association of rosacea with systemic symptoms that might be suggestive of CTD. Regarding the triggering factors, the sun was the most recognised triggering factor affecting more than half of the total of patients (54%). This was followed by eating spicy food, exposure to cold and hot weather and alcohol consumption, occurring in between 44% to 51% of total patients. These results were consistent with the previous surveys performed by the NRS (59).

2. Positive ANA Patients

There are limited data, but a number of studies indicate a higher incidence of immune abnormalities in patients with rosacea. These include the detection of different types of ANA in the blood and the deposition of immunoglobulins at the basement membrane zone of the skin as detected by DIF.

Salo's series in 1970 (149), studied 27 rosacea patients who were diagnosed between 1964 and 1968. This was a review of those patients with investigations including ANA and skin biopsies for DIF from involved facial skin and uninvolved, but light exposed skin of the forearms. The results showed that 15 patients out of 27 had deposition of a thick band of immunoglobulin of homogeneous or granular pattern at the dermo-epidermal junction in samples taken from affected facial skin, with 4 patients having deposition of these immunoglobulins in the uninvolved skin of the forearms. Only 4 patients had a positive ANA of low titre ranging from 1:5 to 1:20. Out of the 15 patients with positive DIF, 9 patients had no specific joint symptoms. However, none of the patients had sufficient criteria to make the diagnosis of lupus erythematosus. The conclusion drawn from this study was that deposition of immunoglobulins seen on DIF as well as a weak positive ANA titre could be a feature of both rosacea and a form of lupus erythematosus. However, the numbers of patients studied were small, and there was no control group. The number of patients who had positive ANA is low and the titre of positive ANA was also too low. The positive DIF from normal facial skin is common, it is well known that sun-exposed skin has a higher frequency of positive immunofluoresence on biopsy, even in a normal population (150), so the relevance of this finding is unclear in the absence of a control group and the findings from this study are somewhat speculative.

Nunzi et al in 1980 (151), investigated 7 untreated patients (4 males and 3 females) with papulopustular rosacea on the face for a period that ranged from 1 to 4 years. The patients were investigated with both direct and indirect immunofluorescence. The results showed that 5 patients out of 7 had positive immunoglobulin deposition at the basement membrane zone when the samples were taken from the involved area (area A), 2 patients had positive immunofluorescence from the uninvolved light exposed area (area B), and 1 patient was positive from uninvolved, non-light exposed skin (area C). In addition anticollagen antibodies were found in 6 out of 7 patients in area A, 4 in area B and 1 in area C. Also, circulating ANA (IgD, IgM, and IgE) with a homogeneous pattern at a titre of 1:40 was found in 2 patients. The suggestion from this study was that the positive DIF at the basement membrane zone in rosacea patients could be a result of antibodies directed against altered collagen type 4 due to photo-damage. The actinic elastosis, which is usually excessive in rosacea patients, may be the cause of this anti-collagen antibody induction. Also, the anti-collagen activity may account for the telangiectasia as a result of immunological injury to the endothelial cells of the vessel wall. Once again, the study is too small and has no control group. The positive ANA detected in 29% (2 out of 7 patients) and the titre was low but the hypothesis with regard of DIF and anticollagen antibodies is an interesting one. It is also possible that some of these patients had both rosacea and lupus, since a positive immunofluorescent biopsy from non-light exposed, uninvolved skin is usually believed to be diagnostic of lupus.

Manna and Marks in 1982 (152), studied 25 patients with rosacea (14 females and 11 males with a mean age of 48 years) and compared them with 25 matched control subjects for past medical history and family history of autoimmune disorders. Patients were also tested for DIF and ANA. They found that rosacea patients and their first degree relatives had a higher incidence of auto-immune disorders including thyroid diseases, rheumatoid arthritis, and diabetes compared to the control group,

although the difference was not statistically significant because of the small size of the study. In addition 23 patients had biopsies for DIF and 18 patients had deposition of IgM and / or IgG and / or complement at the dermo-epidermal junction and / or in the dermal collagen. They also found that 6 patients had a positive circulating ANA of IgM type. The conclusion from this study was that rosacea patients probably are more predisposed to autoimmune disorders and associated with increased positivity of DIF, which could be related to the altered immunogenicity of their facial dermal collagen and their dermal dystrophy. This study is larger in number than the previous studies and is controlled. Although the positive DIF was found in 72%, the positivity of ANA is only detected in 24%, so again the ANA was not significantly positive in this study and its relationship with rosacea was not confirmed. The combination of these findings with those of Nunzi et al does raise the possibility of an immunological reaction at the basement membrane being a part of rosacea pathology that would merit further study.

Alison Black in 1992 (153), studied the prevalence of rosacea in rheumatic skin disease in a dermatology department – based rheumatic skin disease sub-specialty clinic. She studied 21 patients retrospectively. These patients ranged in age from 22 years to 67 years with a male to female ratio of 1:9.5. There was a history of a facial erythematous rash, diagnosed as CLE, occurring in the 5 years before the study was performed. The review of these patients records showed that 9 patients of the 21 (43%) had a positive ANA test, with insignificant or marginal titres, and on repeating ANA test, all of these patients had insignificant ANA titres. Reviewing the diagnosis of these 21 patients after a full history, physical examination, skin biopsy and new laboratory tests revealed that 16 (76%) patients had symptoms and signs of rosacea, and the remaining 5 (24%) patients had other dermatological disorders including perioral dermatitis, allergic contact dermatitis, seborrheic dermatitis and chloasma. The 16 patients with rosacea were treated with topical and oral antibiotics and 15

patients exhibited 75 – 100% clearing of their facial rash. This study shows how physicians can be misled in their diagnosis of rosacea by a low titre ANA. These findings emphasized the need for knowledge of the standard reference values for ANA testing as well as the need to make a full evaluation of ANA positive patients. Even in patients with a confirmed diagnosis of lupus, not all skin disease is necessarily a manifestation of the lupus.

Anna Woźniacka in 2013 (154), in a prospective study investigated 101 rosacea patients with regard of their ANA test and 26 controls matched in sex and age. Over a half of rosacea patients (53.5%) had ANA positive with titer of ≥ 1:160. Within this group 13.86% had a titer of 1:320, 8.91% had a titer of 1:640, and 6.93% had a titer of 1:1,280 or higher. The elevated ANA titers were present more often in women (55.8%) than in men (44.15%). Only 2 of 26 healthy controls had elevated ANA titers, one had a titer of 1: 160 and the other of 1:320. During a two year observation study period, after the initial ANA testing, none of the patients with ANA titers above 1:640 developed an apparent autoimmune disorder. The conclusion from this study was that the elevated ANA titers were commonly found in rosacea patients, and this is with simultaneously existing facial erythema and photosensitivity might lead to misdiagnosis of lupus erythematosus. Clinicians should be aware of these findings to avoid misdiagnosing lupus erythematosus in rosacea patients with elevated ANA titers. This is a large study in terms of number of patients, and was controlled. The positivity of ANA among those rosacea patients was significant (53.5%) and the titre was high of ≥ 1:160 which indicate that there might be true relationship between ANA and rosacea. These figures are very high in comparison with the previous studies, certainly suggestive of a relationship. These findings would ideally be confirmed in a similar national group, since it may be a feature that is unique to this population. It certainly requires confirmation.

So, the above studies showed variable data, indicating a range of views between there being no relationship (Black et al), and a very significant relationship, with over half the patients with the disease having an ANA titre of over 1:160 (Wozniacka et al). Whilst the latter study is extreme and is certainly an outlier compared to most studies, the literature does provide an appropriate basis for this study, particularly given the two very different recruitment centres.

In the study performed for this thesis, all the patients both in Doncaster and Leeds had the ANA blood test performed, however the titre was not recorded. Nevertheless, the rules of the testing laboratories ensured that only titres of ≥ 1:80 were reported as positive. Whilst there is some basis for the value of higher ANA titres being more likely to represent CTD than lower titres, there is also general agreement that an ANA titre of ≥ 1:80 represents a positive test. The purpose of the study was to identify patients with rosacea with a positive ANA at a level that would prompt further investigation, and would be accepted as a positive test in someone with a real CTD. The level of ≥ 1:80 is the level accepted by both of the localities involved in the study. Whilst an investigation of titres would have been interesting, it would not have had a great impact on interpretation in this study, mainly because the overall levels of positivity were low, and the majority of patients with a positive test were already known to have CTD.

Only 22 patients (13%) out of total 169 rosacea patients had a positive ANA (6 patients from Doncaster and 16 from Leeds). All the 6 patients from Doncaster and 13 patients from Leeds were females and only 3 patients from Leeds were males. The numbers of patients with a positive ANA was greater among the Leeds patients probably because patients were recruited predominantly from a specialised clinic for skin and CTD. There is only 1 patient from Doncaster out of the 6 positive ANA patients had a history of lupus and 11 patients from Leeds out of 16 ANA positive

patients had a diagnosis of lupus already made. Another 5 patients from Leeds had rheumatoid arthritis, one had dermatomyositis and one had systemic sclerosis. Some Leeds patients had history of more than one CTD. In the patients without a history of CTD, the percentage of the patients positive for ANA was only 5% and was equivalent in both hospitals. None of these patients were found to have an underlying CTD. This level of positivity was representative of the range of positive ANA tests recorded in the normal healthy general population both from the local laboratories (unpublished data) and in the published literature (140 - 145).

It is the case therefore, that this study did not support the hypothesis that uncomplicated rosacea is related to higher rates of ANA positivity. In a general dermatology clinic, there is also no relationship to an excess of CTD. In addition, the analysis of the subtypes of rosacea with a positive ANA and a history of CTD did not show any specific relationship when the group is taken as a whole. These overall results indicate that a positive ANA alone is found in the same proportion of subjects with rosacea as is seen in the general population. As in other circumstances, the finding of a positive ANA should not of itself lead to the diagnosis of a CTD without a full evaluation, probably including a skin biopsy. However, rosacea and CTD may co-exist. From the data presented here, patients will have additional symptomatology, will usually have used topical, oral or inhaled steroids, and will probably have a PPR pattern to their rosacea. Great care is needed in these patients to make an accurate diagnosis, to ensure appropriate treatment. In particular, if a facial rash is to be used as a diagnostic criterion for lupus, histology will be an essential part of the work up of the patient.

3. Effect of Oral Antibiotics on ANA

Tetracyclines are used orally in the treatment of moderate to severe inflammatory acne vulgaris and rosacea, because of their effectiveness, favorable dosing characteristics and the relatively low rate of resistance. All tetracyclines may be associated with the risk of inducing or precipitating ANA positivity, but this is most marked for Minocycline, where it can occur in up to 15% of patients (155). The association between minocycline and drug induced systemic and cutaneous LE has been extensively documented. Minocycline exposure results in a three-fold increased risk of developing either the systemic or cutaneous form, with a longer duration of exposure correlated with increased incidence. The degree of risk associated with other tetracyclines is less clear, but is certainly very much less (156). Consequently, minocycline has fallen out of favour in this context, but the other tetracyclines are still widely used.

Drug induced SCLE and idiopathic SCLE display almost identical morphologic features, although idiopathic SCLE tends to spare the lower extremities in contrast to drug induced SCLE. SCLE occurs most frequently in women in the third and fourth decades of life, although the drug induced form occurs in both genders and typically at an older age. SCLE typically presents with either scaly, erythematous, annular plaques or with papulosquamous lesions. However, more uncommon morphologies, such as exanthematous, pityriasiform, erythrodermic, bullous and poikilodermatous have been described. Lesions usually are restricted to a photosensitive distribution on the anterior chest, upper back, shoulders, and extensor aspects of the arms. Lesions are longer lasting than those observed in ACLE and are non-scarring in contrast to those in DLE. Up to 70% of patients with idiopathic SCLE and drug induced SCLE have anti-Ro/SS-A antibodies, whereas up to 40% have anti-La/SS-B antibodies (157). The anti-histone IgG autoantibodies are traditionally associated

with drug induced LE in up to 75% of patients although this test is uncommonly positive in SCLE. In contrast, up to 70% of patients with idiopathic SLE are positive for anti-double stranded DNA antibodies, whereas less than 5% of patients with drug induced SLE and SCLE are positive (158).

There is no clear consensus on the diagnostic criteria for drug-induced lupus, but it should be expected in patients who do not have a history of idiopathic lupus, who develop antinuclear antibodies, and who have at least 1 clinical feature of lupus after an appropriate duration of drug treatment. The condition should improve with elimination of the offending drug. Most patients experience improvement within eight weeks of discontinuing the offending medication, with anti-Ro titers becoming normal within eight months (159).

Several mechanisms have been suggested for drug induced lupus and include the possibility that a reactive metabolite binds to the class II major histocompatibility antigen and induces an autoimmune reaction analogous to a graft-vs-host reaction. A drug or its potentially reactive metabolites may bind directly to histones and act as haptens, producing an antigenic complex capable of stimulating autoantibody formation. Factors that have been implicated in causing drug induced lupus include use of the drug for long-term therapy, dose dependency, and the presence of a functional group that is easily oxidized to a reactive metabolite. It was hypothesized that the presence of an amino acid side chain in minocycline, which may yield a reactive metabolite, and the absence of such a functional group in the other tetracyclines might explain why drug-induced lupus is observed mainly in minocycline users (160).

The first case report of minocycline and lupus appeared in 1992. However, in 1998, a lupus like syndrome developing in 64 minocycline users was reported. Patients developed positive ANA and elevated ESR. All patients recovered rapidly after drug removal, and antibody levels became normal. In several cases, re-challenge was positive, indicating a causal relationship (161). A case-control study, of 27,688 acne patients aged 15 to 29 years, recorded on the General Practitioners database in the United Kingdom was reported in 2001. Controls were matched to cases on age, sex, and practice. The main outcome was the development of a lupus like syndrome defined as the occurrence of polyarthritis or polyarthralgia of unknown origin, with negative rheumatoid factor or latex agglutination test, positive or unmeasured antinuclear factor and elevated or unmeasured erythrocyte sedimentation rate. The results showed that a single use of minocycline was associated with an 8.5-fold increased risk of developing a lupus like syndrome compared with non-users and past users of tetracyclines combined. The risk of past exposure to any of the tetracyclines was closely similar to non-use. Current use of doxycycline, oxytetracycline, or tetracycline combined was associated with a 1.7-fold increase of risk. The risk increased with longer use (162). In a Leeds study, in 2007, where 69% of a total of 252 patients with acne vulgaris were treated with minocycline antibiotic, it was shown that no statistical difference existed in the prevalence of ANA positivity between patients exposed (13%) and not exposed patients (11%). However, the higher titres of ANA (1/160 or more) were found more in the minocycline treated group (45%) compared to non-treated group (12%) (163).

All the above studies confirmed the increased risk of drug induced LE with the use of minocycline for the treatment of acne vulgaris. To my knowledge, I could not find any study evaluating the mincycline induced LE in treatment of rosacea, and this is probably because minocycline is not the favorite oral antibiotic in rosacea treatment as well as doctors avoiding prescribing minocycline because of the reported side

effects, especially in patients who were tested positive for ANA to avoid the risk of developing the drug induced LE.

In the study reported in this thesis, there was no increase in the positivity of ANA among rosacea patients who were treated with courses of oral antibiotics. Only 15 patients (11%) out of a total of 139 patients treated with oral antibiotics had a positive ANA compared to 7 patients (23%) out of 30 patients who were not treated with oral antibiotics. This was not a significant difference. Patients were treated with a variety of different tetracyline antibiotics including doxycycline, oxytetracycline, minocycline and lymecycline. We did not record the specific type of oral antibiotic used for treatment of our rosacea patients to check the specific antibiotic effect on the positivity of ANA test, however, the overall results indicated that there is no relationship between all antibiotics usage and a positive ANA result.

4. Conclusion

With respect to the questions originally posed in this piece of research, the following conclusions are drawn:

1. In the cohorts of patients studied, what was the frequency of ANA positivity?

The overall frequency of ANA positivity was 5% for those patients without a connective tissue disease. This study confirmed strongly that there is no evidence of an increased incidence of a positive ANA blood test in a large group of patients with rosacea diagnosed clinically. There is also no evidence that any particular clinical sub-type of the disease is associated with a positive ANA.

2. Is there a sub-group of patients with rosacea that has systemic symptoms with or without immunological abnormalities?

Similarly, there was no increase in symptoms suggestive of CTD in the disease overall, or any of its sub-types. Systemic symptoms occurred, but usually in patients with an underlying CTD.

3. Is there any relation between rosacea and its sub-types and connective tissue diseases, particularly lupus erythematosus?

The more interesting elements of the study came from comparing the two cohorts of patients derived from the two recruiting centres. These centres were quite different in their backgrounds. One, Doncaster, was a district general hospital, where patients were seen in a general clinic with no specific special interest. The second, in Leeds, was based in a teaching hospital, and most patients were recruited through more specialist clinics, where even the ‘general’ clinics had a tertiary referral element, and saw mainly complex medical dermatology.

Patients recruited in the Doncaster cohort were more broadly spread across the range of rosacea sub-types, had low levels of ANA positivity compatible with previously published research and low levels of associated CTD. In Leeds, the patients were more likely to have the PPR pattern of disease, had much higher frequencies of CTD, mainly lupus erythematosus, more regularly used steroids (oral, topical or inhaled), and had higher frequencies of systemic symptoms. There were also more with a positive ANA. Many of the patients with these features had a real CTD that was concomitant, as well as their rosacea. Diagnosis could sometimes be made clinically, but confirmation often required biopsy to separate cutaneous lupus, particularly the rosaceous pattern (98) from true rosacea. This diagnosis was usually clear cut on histological grounds.

The unanswered question in this study is whether rosacea occurs more frequently in certain CTD, and if so, whether this is due to treatment with steroid, or for some other reason. The data here are suggestive of the involvement of steroid treatment, because of its frequent occurrence and the papulo-pustular bias of the rosacea. Whilst confusion of the malar rash of lupus with the ETR sub-type of rosacea remains a further issue, in this study, this did not appear to be a major diagnostic problem for the dermatologists involved, although may well be more difficult for non-dermatologists.

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5. Strength of the Study

1. To my knowledge, this is the biggest study in terms of number of patients (169 patients) investigating the association of different subtypes of rosacea with positivity of the ANA blood test, connective tissue related symptoms and CTD.

2. Numbers were high enough to allow appropriate comparisons to be made with statistical strength.

3. Recruitment from two very different centres allowed for interesting comparisons to be made, particularly since one had a strong bias towards complex medical dermatology and CTD.

6. Limitations of the Study

1. Recruited patients had all had a blood test for ANA performed. This implied that there was at least some possibility in the clinician's mind that an alternative diagnosis might be possible. Mild or frankly pustular clinical patterns might be under-represented since it is these categories of patients in whom an alternative diagnosis is less likely to have been considered. Certain sub-groups e.g. ocular rosacea might also be under-represented since cutaneous disease was a necessary inclusion criterion and is not always present in those with eye disease.

2. All rosacea patients with ANA blood test were recruited retrospectively as there was no budget for this study to investigate ANA or DIF study prospectively in newly diagnosed rosacea patients. Because of this reason the ethical approval was given on basis not to investigate rosacea patients for the purpose of the study.

7. Weakness of the Study

1. Histology and DIF was not performed on all patients, being requested only as clinical need required. It is theoretically possible that some cases of either lupus or rosacea were consequently misdiagnosed. However, our study was retrospective and observational, and rosacea in most instances is diagnosed clinically. Any occurrence of misdiagnosis is likely to have been very limited, particularly since all patients had been tested for ANA. The study also was not ethically approved to investigate any patients further by performing including blood tests or histology on skin tissue prospectively.

2. We did not record the titre of the ANA in our positive ANA patients. The laboratory cut off for reporting positivity was ≥ 1:80, a lower level than has been previously accepted as being clinically important (1:160) in some investigations. However, with modern automated techniques in most laboratories, and certainly in the two involved in performing the ANA screening in this study, the 1:80 cut off is accepted as being positive for the screening nature of the test, and sufficient to prompt further clinical assessment and investigation. Collecting data on titres would have been an interesting addition to the study but is unlikely to have unduly influenced the results.

3. We did not record Anti-DNA, ENA or any of the inflammatory blood markers including C Reactive Protein (CRP) or ESR to correlate these markers with positivity of the ANA. None of the patients with rosacea alone in the Leeds cohort had any antibodies other than the ANA, whilst those with associated CTD had an array of antibodies compatible with their underlying diagnosis. These were not specifically recorded, since they were related to the underlying CTD. Collection on data relating to additional antibodies would have been very important had there been a relationship of ANA to rosacea and its sub-types, but this relationship was not

identified in this study.

4. We did not record the specific type of oral antibiotic used for treatment of our rosacea patients and then correlate this antibiotic with positivity of ANA. The reason was that not all patients had full record of their antibiotics prescribed by their general practitioners documented in their referral letters or hospital notes. However, the overall results indicated no relationship between antibiotic usage and a positive ANA result.

8. Recommendations

1. Further studies are still needed to investigate particularly the frequency of rosacea in a connective disease population. A community based study, identifying patients from a primary care setting with a diagnosis of rosacea, would be valuable to assess the frequency of ANA positivity in this unselected group. There would be a need for the confirmation of the diagnosis, either by an experienced dermatologist, or through skin biopsy, but this would allow the most accurate data to be acquired.

2. A further prospective controlled hospital based study investigating rosacea patients with ANA to establish the frequency of anti-DNA and ENA antibodies in this group would be helpful. A study looking at the frequency of positive DIF in an adequately powered investigation of rosacea patients compared to a healthy control group is recommended, although this would need to be large enough to allow for the relatively high frequency of non-specific DIF positivity in light exposed normal facial skin, as well as the frequency of around 5% ANA positivity in healthy individuals.

3. More studies needed to check the role of the cathelicidin LL-37 in pathogenesis of rosacea and potentially in cutaneous lupus as well. This is particularly important since it may provide a target for therapeutic intervention in these diseases.

4. The role of the DF in the pathogenesis of rosacea is another area of study and the eradication of DF by topical and oral ivermectin to help to control rosacea symptoms especially in the PPR is worth further research.

5. The impact of steroid usage, whether oral or topical is more important, and the balance of rosacea sub-types would also be important areas of further study.

6. The possibility of a role for genetic predisposition in rosacea as a whole, particularly in the light of a positive family history of rosacea, should be further investigated. A simple HLA study or more complex investigations of the genetics of vascular factors, pro-inflammatory cytokines and the innate immune system would be valuable.

Chapter Five - References

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Chapter Six – Appendix

1. **Leeds (Central) Research Ethics Committee**
2. **Doncaster Hospital Ethical Approval**
3. **Leeds Teaching Hospital R&D Approval**
4. **Tables**
5. **Figures**

1. Leeds (Central) Research Ethics Committee

Room 23, Floor CD

Block 40 King Edward Home

Leeds General Infirmary

LS1 3EX

Telephone: 0113 3923772

Facsimile: 0113 3922863

21 April 2009

Dr. Mustafa Marai

Associate Specialist, Dermatology

Dermatology Department,

Doncaster Royal Infirmary & Bassetlaw Hospitals NHS Foundation Trust

Armthorpe Road

DN2 5LT

Dear Dr. Marai

Full title of study:	**A clinical and Pathological Investigation of Rosacea**
REC reference number:	**09/H1313/3**

Thank you for your letter of 15 April 2009, responding to the Committee's request for further information on the above research and submitting revised documentation. The further information has been considered on behalf of the Committee by the Vice-Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA). The favourable opinion for the study applies to all sites involved in the research. There is no requirement for other Local Research Ethics Committees to be informed or SSA to be carried out at each site.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission at NHS sites ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

The participant information sheets and consent forms need to be given validation numbers (3). This will also need to correspond to the text in the consent form referring to the participant information sheet.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Letter of Invitation - LTHT	2	04 March 2009
Letter of Invitation - Doncaster	2	04 March 2009
Response to Request for Further Information		02 March 2009
Interview Schedules/Topic Guides	1	04 March 2009
CV of supervisor		30 December 2008
GP/Consultant Information Sheets		07 January 2009
Compensation Arrangements		02 October 2008
Statistician Comments		
Letter from Sponsor		16 December 2008
Protocol		07 January 2009
Investigator CV		31 December 2008
Application		31 December 2008
Response to Request for Further Information		15 April 2009
Participant Consent Form: Leeds Teaching Hospitals	3	15 April 2009
Participant Consent Form: Doncaster and Bassetlaw Hospitals	3	15 April 2009
Participant Information Sheet: Leeds Teaching Hospitals	3	15 April 2009
Participant Information Sheet: Doncaster and Bassetlaw Hospitals	3	15 April 2009

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website. After Review You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website. The attached document “After ethical review guidance for researchers” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments

- Progress and safety reports

- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

09/H1313/3	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Dr Margaret L Faull **Chair**

Email: rachelt.bell@leedsth.nhs.uk

Enclosures:	**"After ethical review – guidance for researchers"**
Copy to:	**Rachel de Souza**
	R&D office for Leeds Teaching Hospitals Trust

2. Department of Clinical Audit, Research & Effectiveness
Doncaster & Bassetlaow Hospitals NHS Foundation Trust

Tel: 01302 366666 Extension 3820
Fax: 01302 553187

Email: katie.smith@dbh.nhs.uk

18 June 2009

CONFIDENTIAL

Dr. Mustafa Marai

Doncaster Royal Infirmary Hospital

Dermatology Department

Armthorpe Road

Doncaster, DN2 5LT

Dear Dr Marai

Re: A clinical - pathological investigation of rosacea with particular regard to systemic diseases

I am pleased to inform you that the above project has now been given authorisation to commence within Doncaster & Bassetlaw Hospitals NHS Foundation Trust. For your information, the project reference is 207/2008/STU. I would be grateful if you could quote this number in any further correspondence with this department.

Documentation

Your authorisation has been granted based on submission of the following documentation:

1. Research Protocol 8171/19699/1/523

2. IRAS/REC form (Signed by Mrs Rachel DeSouza, 1 May 2009)
3. SSI form (Signed by Dr Mustafe Marai 10 May 2009)
4. Sponsorship statement (University of Leeds, 29 April 2009)
5. Letter stating 'favourable ethical opinion' from Leeds (Central) Research Ethics Committee (21 April 2009)
6. Indemnity statement (AIG Europe (UK), letter from Marsh, 2 October 2008)
7. Study data proforma (22 April 2009)
8. Invitation to participation in Rosacea Study (22 April 2009)
9. GP information letter (22 April 2009)
10. Consent form (22 April 2009)
11. Rosacea Study Participation Information Sheet (22 April 2008)
12. Patient Information sheet and questionnaire (all documents) with version and date
13. MHRA approval with date
14. Clinical Trial Agreement with version and date

Please note that approval is limited to the dates stated on the research application form and that you are obliged to notify the R&D Department of any adverse events that arise during the course of the project. You are also obliged to inform us if your project deviates in any way from the original proposal / documentation you have submitted. This may result in the suspension of your project until changes have been agreed with the Trust.

Permissions

This letter authorises you in principle to undertake research within the Trust. However, it is your responsibility to ensure that individuals appropriate to your work have no objections to your studies. This department accepts no liability for non co-operation of staff or patients.

Contracts

It is your responsibility to ensure you have sufficient indemnity to undertake this project and that letters of authority / honorary contracts are in place where necessary.

Auditing

I would strongly urge you to maintain an accurate and up to date site file for your documentation, as the Trust randomly audits projects to assess compliance with the relevant frameworks and legislation. If your study is chosen, you will be notified in writing not less than two weeks prior to the required submission date of documentation.

May I take this opportunity to wish you well with your project. If you have any questions or I can be of any further assistance to you, please do not hesitate to contact me.

Yours sincerely

Emma Hannaford

Research Governance Co-ordinator

CC Local Investigator

3. Leeds Teaching Hospital R&D Approval

Dr Mark Goodfield

Consultant Dermatologist

Dermatology Department

Leeds General Infirmary

31/07/2009

Dear Dr Mark Goodfield

Re: LTHT R&D Approval of: A clinical and Epidemiological Investigation of Rosacea with particular regard of systemic symptoms

LTHT R&D Number: EX09/8870

LREC: 09/H1313/3

I confirm that this study has R&D approval and the study may proceed at The Leeds Teaching Hospitals NHS Trust (LTHT). This organisational level approval is given based on the information provided in the documents listed below.

In undertaking this research you must comply with the requirements of the Research Governance Framework for Health and Social Care which is mandatory for all NHS employees. This document maybe accessed on the R&D website;

http://www.leedsth.nhs.uk/sites/research_and_development/

R&D approval is given on the understanding that you comply with the requirements of the Framework as listed in the attached sheet “Conditions of Approval”. If you have any queries about this approval please do not hesitate to contact the R&D Department on telephone 0113 392 2878.

Indemnity Arrangements:

The Leeds Teaching Hospitals NHS Trust participates in the NHS risk pooling scheme administered by the NHS Litigation Authority 'Clinical Negligence Scheme for NHS Trusts' for; (i) medical professional and/or medical malpractice liability; and (ii) general liability.

NHS Indemnity for negligent harm is extended to researchers with an employment contract (substantive or honorary) with the Trust. The Trust only accepts liability for research activity that has been managerially approved by the R&D Department.

The Trust therefore accepts liability for the above research project and extends indemnity for negligent harm to cover you as principal investigator and the researchers listed on the Site Specific Information form. Should there be any changes to the research team please ensure that you inform the R&D Department and that s/he obtains an employment contract with the Trust if required.

Yours sincerely

Dr D R Norfol

Associate Director of R&D

4. List of Tables

5. List of Figures