British Journal of Dermatology (1993) **128**, 650–659.

Density of *Demodex folliculorum* in rosacea: a case-control study using standardized skin-surface biopsy

F.FORTON AND B.SEYS*

Clinic of Dermatology, Saint Pierre University Hospital, Université Libre de Bruxelles, Brussels, Belgium
**Unit of Medical Sciences Pedagogy, Faculty of Medicine, Université Catholique de Louvain, Brussels, Belgium*

Accepted for publication 26 June 1992

Summary A standardized skin-surface biopsy (1 cm^2) of the cheek was performed in 49 patients with rosacea [13 with erythematotelangiectatic rosacea (ETR), three with squamous rosacea (SR), 33 with papulopustular rosacea (PPR)], and 45 controls.

A mean density of 0·7 *Demodex folliculorum*/cm^2 was found in controls, 98% of whom had less than five *Demodex*/cm^2. When all clinical types of rosacea were considered collectively, the density of *Demodex* was significantly higher in patients with rosacea than in controls (mean = 10·8/cm^2; $P<0·001$). When the various clinical types of rosacea were considered separately, *Demodex* density was statistically significantly higher than in controls only in the PPR patients (mean = 12·8/cm^2; $P<0·001$).

The same type of comparison was also made for three other groups of subjects—patients with isolated inflammatory papules ($n=4$), rhinophyma ($n=3$), and HIV infection ($n=21$), respectively: in these groups, the *Demodex* density did not differ significantly from controls.

The present study demonstrates a high density of *D. folliculorum* in PPR, and supports its pathogenic role in the papulopustular phase of rosacea. The study suggests that standardized surface biopsy could be a useful diagnostic tool for PPR, with a 98% specificity when *Demodex* density is higher than 5/cm^2.

Demodex folliculorum is a transparent mite, 0·3 mm long, which asymptomatically parasitizes the human pilosebaceous follicles (Fig. 1).[1–4] The proportion of *Demodex* carriers (prevalence) increases with age,[1,5–9] and the reported prevalence is also determined by the fastidiousness of the detection method used.[5–17] A variety of prevalence rates in different age-groups have been reported in a number of studies (Table 1).[5–7,11,14]

The pathogenic role of *Demodex* is still a matter of debate. To date, it has been implicated in the occurrence of the dry, 'pityriasis-like' erythematosquamous rosacea,[18] in papulopustular and/or granulomatous rosacea,[19–22] including localization on the bald scalp,[23] in isolated inflammatory papules,[13,21,24] and in some cases of blepharitis.[25]

It is difficult to establish the pathogenicity of *D. folliculorum* in rosacea because of three factors: the localization of the disease, the obligate character of the parasite, and its ubiquity in man. Indeed, because rosacea is a benign dermatosis mainly localized to the face, it may be difficult to justify a standard skin biopsy to study the disease. In addition, as *D. folliculorum* is a very host-specific obligate parasite, it cannot at present be grown *in vitro*, which makes a massive experimental infestation impossible. The mere detection of its presence is no proof of pathogenicity because it is so ubiquitous. It is usually considered as playing a pathogenic role when present in 'very large' numbers,[18] and in an intradermal location.[19–21,24]

The principal aim of the present study was to determine the pathogenic relevance of *D. folliculorum* in rosacea by comparing the mite density in healthy subjects with that in patients suffering from rosacea. We considered the various clinical types of rosacea separately, i.e. erythematotelangiectatic (ETR), squamous or pityriasis-like rosacea (SR), and papulopustular rosacea (PPR).

In addition, the same comparison was made in patients suffering from rhinophyma or isolated inflammatory papules (IIP), and in individuals suffering from human immunodeficiency virus (HIV) infection.

Correspondence: Dr F.Forton, rue F. Binjé 8, B-1030 Bruxelles, Belgique.

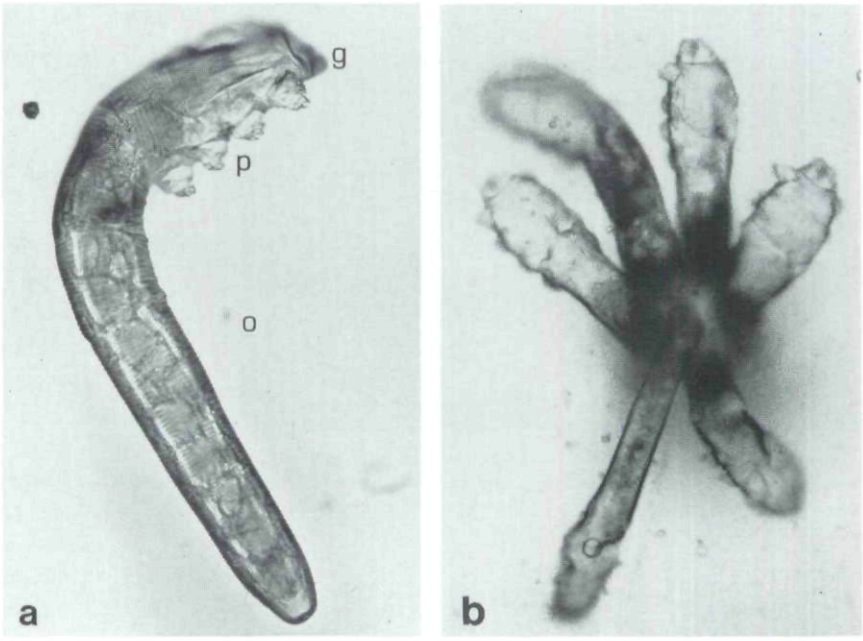

Figure 1. *Demodex folliculorum* as it can be seen on a skin surface biopsy. (a) Three-quarters right aspect (× 500): from top to bottom, the body consists of a gnathosome (g) or rostrum made of the oral parts, a podosome (p) bearing four pairs of legs, and an opisthosome (o) or abdomen, transversely striated along its whole length. (b) Six parasites grouped in the same pilosebaceous follicle (× 250).

Table 1. *Demodex* prevalence according to sampling methods since 1910*

Reference		Method	Subjects			Prevalence %
			n	State	Age (years)	
Dubois[5]	1910	Nose, comedone expression	250	a	<5	0
				a	5–10	50
				a	>10	100
Kaufmann-Wolf[10]	1925	50 lanugo hairs, epilation	10	a	30–57	90
Fuss[11]	1933	Comedone-extractor, 3 successive extractions	100	a	1–82	100
			—	c	—	100
Beerman and Stokes[12]	1934	Comedones and superficial scrapings	29	a	—	52
Breckenridge[13]	1953	1 slide/biopsy, 1 biopsy/case	1435	a	—	13
Riechers and Kopf[14]	1969	16 biopsies/case	9	c	53–91	100
Norn[6]	1970	50–150 lashes/case, epilation	100	c	40–100	89
		8 lashes/case, epilation	400	a	40–69	48
Norn[1]	1971	280 hairs, epilation; or expression of material from sebaceous glands	50	a	44–96	98
Nutting and Green[15]	1976	Biopsy	23	a	21–60	70
Roth[7]	1979	Eyelid biopsy, multiple step sections	100	a	27–87	84
Aylesworth and Vance[8]	1982	Biopsy	1124	a	0–90	10
		Biopsy exhibiting follicles	668	a	0–90	18
Norn[16]	1982	Adhesive tape impression, nose and eyelids	206	a	0–80	28
Andrews[9]	1982	1 single sebum extraction from nasolabial folds	88	a	19–58	17
Sengbusch and Hauswirth[17]	1986	1 single sebum extraction from nasolabial folds	370	a	0–90	54·9

* For references prior to 1910, see Beerman and Stokes[12]; a, alive, c, cadaver.

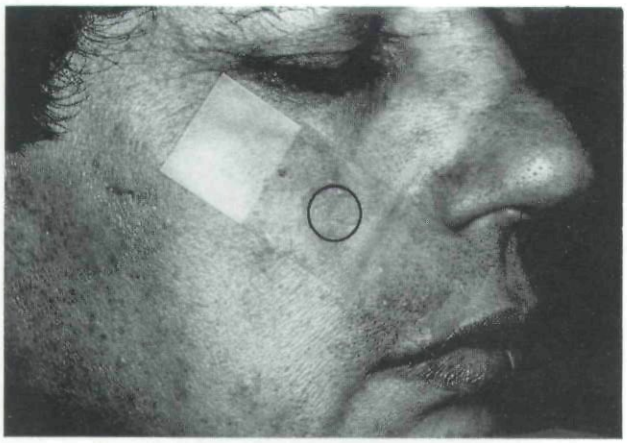

Figure 2. Standardized skin-surface biopsy. A slide with, on the skin side, a drop of cyanoacrylic adhesive, and, on the other side, a black circle enclosing a standard area of 1 cm^2, is applied to the skin.

Methods

Sampling method: the standardized skin-surface biopsy

Skin-surface biopsy is a non-invasive sampling method by which it is possible to collect the superficial part of the horny layer and the complete follicle contents. It consists of placing a drop of cyanoacrylic adhesive (Loctite®) on a microscope slide, applying the adhesive-bearing surface of the slide to the skin, and removing it gently after it has been allowed to dry (about 1 min).[26,27] Initially, a black preprinted circle, 11·5 mm in diameter (Decadry® No. 223, Alphac Products), is placed on the slide, to facilitate examination of a standard surface area of 1·03 cm^2 (Fig. 2). After removal from the skin, each sample is clarified with 2–3 drops of immersion oil, and then covered with a cover slip.

The samples were studied microscopically at standard magnifications (×40, ×100, ×400), and each specimen was examined at least three times. The examination was performed as soon as possible after sampling, in order to aid detection of the parasites, because their movements decrease with time, and they gradually disintegrate. In the present study, a maximum of 4 h elapsed between the time of sampling and examination of specimens.

Only *D. folliculorum* clearly identified on the basis of anatomical characteristics were counted.[4] A detailed record of the number and distribution of the mites was kept on a chart in the patient's notes. The cheek was the site used for skin surface biopsy in controls, and in patients with rosacea, and HIV infection. In cases of rhinophyma, the biopsy was on the nose, and in patients with inflammatory papules on the face, samples were taken from directly over the lesions. The clinical and microscopic examinations were performed by the same investigator.

Patients

All patients in the study gave informed consent to skin-surface biopsy. They were recruited from in-patients and out-patients at the Saint Pierre and Brugmann University Hospitals in Brussels (Belgium), from the staff of these hospitals, and from close relatives of the authors, between March 1987 and November 1988. Eleven patients were excluded from the study at the time of analysis: four with ETR, five with PPR, one with IIP, and one HIV-seropositive patient. The reasons for exclusion were as follows: the interval between sampling and microscopic examination exceeded 4 h or was not known; microscopic examination had not been performed; one HIV-seropositive patient was excluded because his staging was not known.

Five groups of patients were analysed.

1 Forty-five controls with normal facial skin, without telangiectases (mean age 41 years; SEM 2·3; range 21–79). Thirty-four (75%) were women.

2 Forty-nine patients with rosacea: 13 ETR, 3 SR, and 33 PPR (mean age 49 years; SEM 1·8; range 22–78). In the subgroups, the mean age was 49, 58, and 48, respectively. Thirty-two patients (65%) were women. The proportion of women in the various subgroups was almost identical (77% ETR; 67% SR; 61% PPR). The controls and the rosacea patients were matched for sex, but not for age ($P<0·01$).

In the 33 PPR patients, the nature and duration of previous therapy were noted. Eight patients had not been treated, or their previous therapy had been discontinued at least 2 months before the first visit; five were receiving cosmetic treatments (moisturizing creams); seven were being treated with topical steroids; nine were receiving other treatments, including oral tetracyclines, antibiotic creams, and topical epinephrine hydrochloride; in four the type of treatment was not known.

3 Four female patients suffering from IIP (mean age 52 years; SEM 6·4; range 35–65). The papules were localized periorally (one patient), on the cheek (one patient), the chin (two patients), and the nose (one patient).

4 Three male patients with rhinophyma who were aged 65, 71, and 77.

5 Three factors led us to analyse the density of *D. folliculorum* in the patients with HIV infection: (a) the

Table 2. *Demodex* density in the groups studied

Studied groups	*n*	*Demodex* density (*Demodex*/cm²) Median	Q3–Q1	Mean ± SEM	Tests
Controls	45	0	1	0·7 ± 0·25	—
Rosacea (all)	49	4	18	10·8 ± 2·02	$P<0·001$
Erythematotelangiectatic	13	0	1	5·1 ± 4·26	NS
Squamous	3	13	27	13·3 ± 7·80	NS
Papulopustular	33	7	20	12·8 ± 2·36	$P<0·001$
Rhinophyma	3	0	—	0	—
Isolated inflammatory papules	4	0	17	6·3 ± 4·05	NS
HIV seropositive					
Stage I	2	0	—	0	—
Stage II	1	0	—	0	—
Stage III	4	0	—	0	—
Stage IV	14	0	5	1·8 ± 0·81	NS

n, number of subjects; Q3–Q1, interquartile interval; tests, comparison with controls by robust rank-order test; NS, not significant; —, no computable value.

development of opportunistic infections in immunodepressed patients, including AIDS patients; (b) two reports of an increase in numbers of *D. folliculorum* during corticosteroid therapy;[28,29] (c) the fortuitous finding by a colleague of a large number of *D. folliculorum* in a sample of pityriasis versicolor scales taken from the thorax of an AIDS patient (this patient did not participate in the study).

Most of the 21 HIV-positive patients were male [$n=14$ (67%)], which differentiates them from controls ($P<0·005$). Their disease stage was determined during the clinical part of the study according to the nomenclature used at the time: two were asymptomatic carriers (stage I), one was in stage II (lymphadenopathic syndrome), four in stage III [AIDS-related complex (ARC)], and 14 in stage IV (full-blown AIDS). The mean age of these patients was 37 (SEM, 2·4; range, 22–60).

Statistical analysis

Comparability of control and study groups for sex and age was assessed by means of the chi-squared test and Student's *t*-test, respectively.

Because *D. folliculorum* densities are counts, these are likely to follow a priori a Poisson distribution, and the variance will alter with the mean. This was actually observed in control and study groups (Table 2). In such a situation, the usual parametric or non-parametric techniques which are sensitive to the equality of variances of the underlying distribution are not useful. Two methods were used to overcome this difficulty, known as the Behrens–Fischer problem.[30] (i) The dependent variable (*D. folliculorum* density) was applied a square-root transformation in order to obtain an equalization of variances. An ANOVA technique was then performed on these data.[31] This method was used for testing the relationship between *D. folliculorum* density and previous therapy in the PPR group. (ii) Because intragroup variances remain too unequal, despite this transformation, to test the main hypothesis of this study (Table 2) and relationship to age (Table 3), two non-parametric techniques were used which require no assumption on the underlying distribution of the data: the robust rank-order test for comparison of *D. folliculorum* density

Table 3. Association between *Demodex* density and age in controls and rosacea groups

Age-groups (years)	Controls *n*	Controls *Demodex* density Mean ± SEM	Rosacea *n*	Rosacea *Demodex* density Mean ± SEM
20–29	15	1 ± 0·7	4	15·5 ± 8·9
30–39	10	0·1 ± 0·1	6	5·3 ± 3·3
40–49	8	1·3 ± 0·5	17	17 ± 4·6
50–59	7	0·4 ± 0·3	13	7·3 ± 2·8
60–69	0	—	7	5·6 ± 1·8
70–79	5	0·8 ± 0·8	2	5·5 ± 2·5
r_s value		0·028		−0·066
Test		NS		NS

n, number of subjects; *Demodex* density, *Demodex*/cm²; r_s, Spearman rank-order correlation coefficient; —, no computable value.

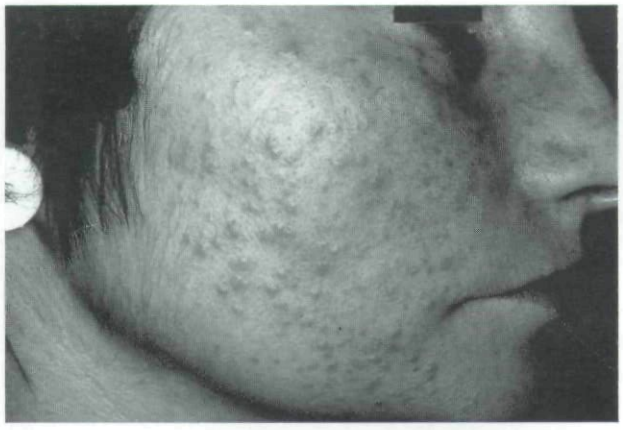

Figure 3. PPR in a 33-year-old woman in whom skin-surface biopsy showed six *Demodex*/cm^2.

between each of the studied groups and controls, and the Spearman rank-order correlation coefficient (r_s) for analysing the association of age and *D. folliculorum* density both in control and rosacea groups (Table 3).[30]

Tests were performed at 0·05 significance level. Rejection region was two-tailed, except for analysis of age (Table 3) in which it was one-tailed, in accordance with the hypothesis of an increased *Demodex* density with age.

Mean values are given with the standard error of the mean (SEM). When consistent, the median and the interquartile interval (Q3–Q1) are given.

Results

The density of *D. folliculorum* showed no significant relationship with age, either in the control group or in the rosacea group (Table 3).

Table 4. Value of the criterion 'density of *Demodex folliculorum* > 5/cm^2' to confirm a clinical diagnosis of papulopustular rosacea (PPR)

Density of *D. folliculorum*/cm^2	PPR	Controls	Total
>5	18	1	19
<5	15	44	59
Total	33	45	78

Diagnostic value: specificity 44/45 = 0·98;
sensitivity 18/33 = 0·55;
positive predictive value 18/19 = 0·95;
negative predictive value 44/59 = 0·75.

The density of *D. folliculorum* in the group of rosacea patients (mean 10·8; median 4) was significantly higher than in controls (mean 0·7; median 0) [$P<0·001$] (Table 2).

Demodex folliculorum density in the PPR patients (mean 12·8; median 7) was significantly higher than in controls (mean 0·7; median 0) [$P<0·001$] (Table 2; Figs 3 and 4).

In the ETR, SR, IIP and stage IV AIDS groups, the density was higher than in controls, although none of these differences was significant (Table 2). In IIP patients, the observed densities were 17, 8, 0 and 0. The density was nil among rhinophyma and stage I, II, and III AIDS patients.

When compared with controls, a density of *D. folliculorum* > 5 was very PPR-specific. This criterion had a 98% specificity, a 95% positive predictive value, a 55% sensitivity, and a 75% negative predictive value (Table 4).

The scattering of *D. folliculorum* densities varied markedly according to the group studied (Table 2). They were not very scattered in the controls and ETR groups, where 50% of the values around the median (interquartile interval) spread on an interval of only 1 *Demodex*/cm^2. The values were more scattered in the PPR group, where the interquartile interval was 20, and in the SR group, where the interval was 27 (however, the small number of findings in the latter group did not allow any conclusion to be drawn). In the other groups, *D. folliculorum* densities showed an intermediate scattering pattern.

In the PPR group, there was no significant relationship between previous therapy and mean *D. folliculorum* density, although the values varied from 6·9/cm^2 in cases treated with topical corticosteroids to 19·6/cm^2 in those receiving no treatment. The values were intermediate in cases receiving cosmetic treatment (16·4 ± 7·3/cm^2) or other treatments (11·4 ± 3·8/cm^2).

Discussion

Demodex (Greek: *demos* = fat; *dex* = woodworm) was first discovered by Henle and Berger in 1841, and described in detail by Simon in 1842. Two different species can be found in man: *Demodex folliculorum*, a long form which lives in the pilosebaceous duct, and *Demodex brevis*, a short form which inhabits the sebaceous and meibomian glands.[1]

The real prevalence of the mite is probably 100%, based on evidence from the most fastidious studies which

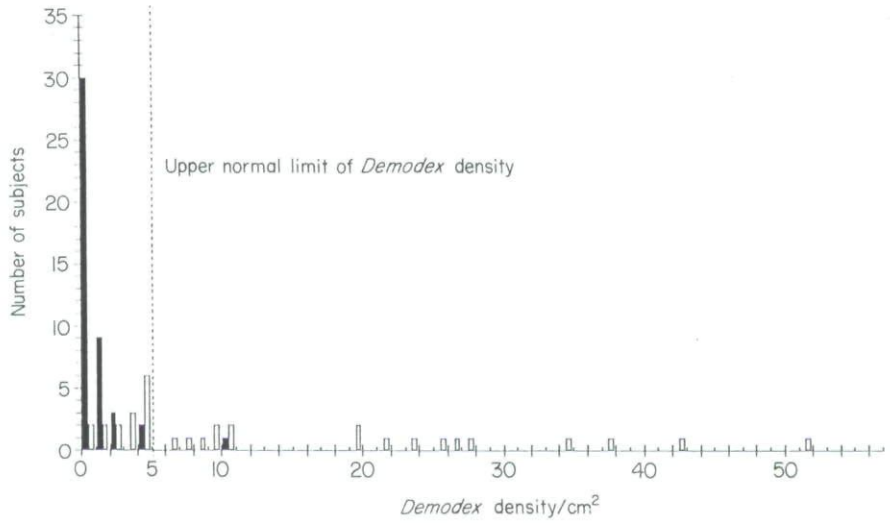

Figure 4. Distribution of *Demodex* density in control and PPR groups. ■ controls ($n=45$); □ PPR ($n=33$).

have employed many samplings per individual (Table 1).[5,11,14] However, we found only 15 *Demodex* carriers in 45 controls (i.e. a prevalence of 33·3%), but this was probably because we sampled only a small area on each subject's face. Standardized skin-surface biopsy is not a method designed to study the mite prevalence in the population, but to estimate *Demodex* density—or, more precisely, *Demodex folliculorum* density—in each subject. The method collects the superficial part of the horny layer and the whole follicle contents, and therefore detects the few mites present on the skin surface and the more numerous mites in the pilosebaceous duct,[16,32] i.e. almost exclusively *D. folliculorum*.[2,32] *Demodex brevis*, which is principally found in the sebaceous glands,[2] and the occasional *D. brevis* and/or *D. folliculorum* which have penetrated into the dermis are not detected by this method.

Most published observations have shown that the prevalence of *Demodex* increases with age (Table 1).[1,5–9] In a more recent study, Sengbusch and Hauswirth found a pronounced increase in *D. brevis* prevalence with age, whereas the prevalence of *D. folliculorum* tended to remain more constant.[17] In our study, we did not observe an increased *D. folliculorum* density with age, either in the control group or in the rosacea group (Table 3). This stability of mite density with age could partly explain the stability of its prevalence.

The role of *D. folliculorum* in rosacea remains controversial. Marks and Harcourt-Webster in 1969,[33] and Ramelet and Perroulaz in 1988,[34] studied skin biopsies from patients with rosacea, and found mites in only a small proportion of cases (14/74 PPR[33] and 2/75 rosacea[34]). They suggested that these results argued against a pathogenic role for *Demodex* in rosacea. The discrepancy between these studies and our own observations can be explained by the different methodology employed. *Demodex* is not as easily detected in histological preparations as in skin surface biopsy. In skin-surface biopsy specimens the mites are intact, alive, they move, and are easy to detect, whereas, according to Bøge-Rasmussen, 'in histologic preparations, the mite shrinks rapidly and transforms into a translucent "ghost" sac of chitin which it is impossible to identify in the preparation'.[28] However, with some experience, and a good knowledge of its anatomy,[4] *Demodex* is not very difficult to detect, and this is reflected by demonstration of the mite in 10% of skin-biopsy specimens.[8] Another possible explanation would be that, although standard skin biopsy allows the detection of *Demodex*, it is, however, limited by the small volume of material assessed. A standard biopsy from a patient with rosacea is usually small (<1 cm^2) because it is taken from the face, and only a limited number of sections are examined (for example, four sections/case[34]). In addition, the sections do not necessarily contain a follicle, a necessary prerequisite for detection of *Demodex*.[8] Finally, because the thickness of the section is usually 5 μm, and the mean interval between two sections is 25–50 μm, *D. folliculorum*, the opisthosome of which has a width of 31–44 μm,[3] and runs parallel to the axis of the hair, may be missed because it is present between two sections. Performing serial sections at narrow intervals may, of course, reduce the likelihood of this. In the studies mentioned above,[33,34] serial sections were carried out in only 24 of 108 biopsies (at 20-μm intervals),[33] and in seven of 75 cases.[34] In contrast, the sampling obtained with skin-surface biopsy corresponds exactly with the usual location of *D. folliculorum*, and makes it possible to

analyse easily a large skin sample: a surface biopsy of 1 cm^2 allows detection of all, or nearly all, *D. folliculorum* present in that area, because such a biopsy is composed of both superficial and deep tissue (hair infundibula). This does not, however, apply to *D. brevis*, and the very few *Demodex* which have penetrated into the dermis, which can only be detected by standard skin biopsy. In our opinion, such a difference in the performance of the methods used to detect *D. folliculorum* may largely explain the discrepancy between the studies mentioned above and our own study.

In the studies of Marks and Harcourt-Webster,[33] and Ramelet and Perroulaz,[34] the mite had induced little or no local inflammation around the follicles, and had not penetrated the dermis, but it should be noted that these observations were made on only 14 and two mite-containing biopsies, respectively. However, perifollicular inflammation, and penetration of the dermis by mites, are phenomena which have been extensively documented. Perifollicular inflammation occurring around infested follicles has been noted by several authors, based on systematic examination of skin biopsies (not only of rosacea).[7,8,13,35] In 1979, Roth examined 100 palpebral skin biopsies, and found an infiltrate around 42% of infested follicles.[7] In 1982 Aylesworth and Vance examined 1124 biopsies, 117 of which were infested by *Demodex*, and found a lymphohistiocytic infiltrate around 29% of the infested follicles.[8] In 1986, we examined 69 biopsies (selected only on the basis of the presence of *Demodex*). We found 986 *Demodex* and 710 follicles, and established a statistically significant relationship between the presence of *Demodex* and perifollicular lymphohistiocytic inflammation.[35] Other isolated histological findings are in agreement with this. *Demodex* feeds on epidermal cells,[15] and thus creates breaches in the superficial layers of the follicular epithelium, towards which the infiltrate makes its way, sometimes producing a true *Demodex* folliculitis.[35] The fact that *Demodex* has been observed in biopsies from a variety of skin diseases does not conflict with its involvement in rosacea.

The intradermal presence of *Demodex* has been documented by histopathological studies of granulomatous and/or lupoid rosacea. In very rare instances, *Demodex* may make its way through the follicular wall[35] and induce in the dermis a granulomatous reaction with giant cells which will phagocytose the parasites.[20,21] Grosshans found *Demodex* in the granulomas in 10 of 53 patients with lupoid rosacea.[21] In contrast, Ecker and Winkelmann could find it in only 1 of 30 cases; however, they did not specify whether they performed serial sections in their study.[20] The lymphocytes from the *Demodex* granulomas and the perifollicular infiltrate of rosacea are of the same type, i.e. T-helper lymphocytes.[36] Moreover, anti-*Demodex caprae* antibodies can be found in 22% of patients with PPR and granulomatous rosacea. These antibodies resemble those produced by rabbits sensitized by *D. caprae* antigens.[37]

In 1965, Robinson studied the mite population in 3-mm punch biopsy specimens from the nasal crease, and did not observe any significant change after 28 days of therapy with a 3% sulphur preparation applied to one half of the face, although there was clinical evidence of a beneficial effect from the treatment.[38] As Robinson stated, it was not possible to conclude from the study that treatment with 3% sulphur did not affect the mite counts 'as the method used may not be sufficiently sensitive to detect the difference', because the area sampled was limited to the diameter of the punch (3 mm).

Contrary to the opinion of Marks and Harcourt-Webster,[33] Ramelet and Perroulaz,[34] and Robinson,[38] other authors are in favour of a role for *D. folliculorum* in rosacea. Large numbers of mites were found in pustules in 50 patients with pustular rosacea,[10] in the dry pityriasis-like erythematosquamous rosacea, and in a dry type of PPR (10–15 *Demodex*/'follicular scale'),[18] in 16 of 18 patients with rosacea,[39] in 9 of 10 patients with rosacea (skin surface biopsies),[27] in a pustular folliculitis of the face,[22] and in a unilateral rosacea (15 *Demodex*/low-power field, in samples obtained by scraping).[40] Several studies have demonstrated a decrease in the number of *Demodex* following local treatment (with Danish ointment,[18] with crotamiton,[22,40] or with hexachlorocyclohexane[39]), and this decrease was associated with a dramatic clinical improvement.

In 1925, Kaufmann-Wolf studied 50 cases of pustular rosacea and found the mite in samples from the pustules in all cases, but *Demodex* were not more numerous on hairs taken from the lateral area of the face.[10] This finding might be interpreted as indicating that, in pustular rosacea, mite proliferation is only superficial (at the level of the pustule). However, our study demonstrates an increase of *Demodex* in the pilosebaceous follicles, not merely in superficial pustules. This is probably due to the fact that the hairs observed in our skin surface biopsies were taken from areas of PPR, in contrast with those studied by Kaufmann-Wolf.

In 1981 Varotti *et al.*[41] found *D. folliculorum* in all their patients with rosacea ($n=25$), whereas the mite was absent in all their healthy controls ($n=25$). They used a tape-stripping technique which consisted of scraping the skin several times, and collecting the scrapings by means of double-sided adhesive tape stuck on to a microscope

slide. Following treatment with oral metronidazole (500 mg/day for 20 days, followed by 250 mg/day for 20 days), no *D. folliculorum* could be found on the patients' cheeks. The authors suggested that their method could detect the mite only when it was present in large numbers. They were in favour of a role for *D. folliculorum* in the pathogenesis of rosacea.

Our study confirms in a more accurate way that the density of *D. folliculorum* in the rosacea group (10·8/cm^2) is greater than in the control group (0·7/cm^2). The difference is principally determined by the mite density in the PPR group (12·8/cm^2). The density among the ETR patients (5·1/cm^2) is not statistically significantly different from that observed in controls. Such a result may possibly be due to lack of power of the test [resulting from a small number of subjects ($n=13$)]. However, we are inclined to believe that *Demodex* density among the ETR patients is normal, or intermediate between the controls and the PPR patients, in view of the succession of the clinical stages of rosacea. Further studies involving larger numbers of subjects should clarify this point.

The SR patients apparently had larger numbers of *Demodex* (13·3/cm^2) than controls (0·7/cm^2), but because of the limited number of cases we were not able to demonstrate a statistically significant difference.

In practice, it is of value to know the threshold between a 'normal' and 'abnormal' density of *Demodex*, to be able to confirm the clinical diagnosis of PPR. Therefore, on presentation of the results of our study, we arbitrarily chose a threshold of 5 *Demodex*/cm^2 to obtain a very specific test, with few false-positive results (2%), which therefore becomes less sensitive, i.e. with many false-negative results (45%) [Table 4; Fig. 4]. This is why a density greater than 5 *Demodex*/cm^2 allows us to confirm the diagnosis of PPR with a positive predictive value of 95%. On the other hand, a density lower than, or equal to 5 *Demodex*/cm^2, does not allow us to draw any conclusion, because there is a 25% likelihood that the disease is PPR anyway (negative predictive value = 75%). Some PPR cases have less than 5 *Demodex*/cm^2, but it would be too much to hope for a density lower than, or equal to, 5 *Demodex*/cm^2 in all the controls, and above 5 *Demodex*/cm^2 in all the cases of papulopustular rosacea, as there is no such perfect test in biology. This is probably due to the multifactorial nature of rosacea. The clinical picture of rosacea depends not only on *Demodex* density, but also, for example, on the inflammatory reaction induced by the mite: it is possible that this reaction varies from individual to individual, each subject responding to their own *Demodex* density threshold.

Finally, such a test compares PPR patients with healthy controls, and before it can be used as a differential diagnostic tool in PPR, the *Demodex* densities in other facial dermatoses clinically similar to PPR require study, particularly lupus erythematosus, in order to verify whether they equate with those of controls. Moreover, it would be useful for the proposed threshold density to be validated by other studies on larger patient samples.

From a pathogenetic standpoint, the higher *Demodex* density observed only in PPR suggests, although it does not prove, that *Demodex* plays a role in the formation of the papules and pustules of rosacea only at this stage of the disease (stage III), and not before. This would be compatible with the present concept of the pathogenesis of rosacea, in which the disease is considered as a 'primarily' venous vascular functional disease of the face, possibly originating in poor blood flow control by the central nervous system.[42,43] The vascular changes probably create an environment which favours the multiplication of *Demodex* and/or their penetration into the dermis, and this would then induce macroscopically visible inflammation in the form of papulopustules: such an hypothesis was suggested by Spickett in 1962.[44] However, the multiplication of *Demodex* and/or their penetration into the dermis may occur independently of predisposing vascular factors, thus leading to the formation of IIP. Indeed, two of the four patients with IIP had a high *Demodex* density (17 and 8/cm^2), which suggests that the mite may induce an inflammatory response which falls outside the clinical scope of rosacea, as has already been reported.[7,8,13,21,24,35] In our group of IIP patients, however, the mean *Demodex* density was not statistically different from that of controls; this is probably due partly to the small size of the IIP sample, and partly to the fact that IIP may have many other causes than *Demodex*.

Demodex folliculorum density does not appear to be higher in cases of rhinophyma (mean = 0/cm^2), but there were only three cases of rhinophyma in our study. Clinically, rhinophyma differs markedly from rosacea, and it can develop in the absence of papulopustules. Moreover, it principally affects male patients. It is however, considered as stage IV of the disease. Perhaps it is a different, although closely related, disease. It is also possible that rhinophyma might be more readily associated with a proliferation of *D. brevis* rather than *D. folliculorum*; the absence of *D. brevis* in our study would then be directly related to the technique used.

We did not find any increase of *Demodex* density in the group of patients with HIV infection compared with controls.

The findings of the present study should be confirmed in a larger patient group, particularly with regard to patients with ETR, SR, and controls from an older age-group. A similar study should also be carried out on other facial dermatoses. Standardized skin surface biopsy could be used to monitor the evolution of *Demodex* density in rosacea during treatment, for example with topical metronidazole or crotamiton (Eurax®), each patient serving as their own control, by comparison of treated and untreated sides of the face.

Standardized skin surface biopsy may also be utilized for the study of other dermatological diseases in which it has been suggested that *Demodex* may play a pathogenic role, for example perioral dermatitis,[39,45,46] and Grover's disease.[47]

This study supports the pathogenic role of *D. folliculorum* in PPR and suggests the use of a standardized skin-surface biopsy as a diagnostic tool for the disease. A diagnosis would be considered positive when the density of *Demodex* is above $5/cm^2$.

Acknowledgments

We thank Professor G.Achten, Professor M.Ledoux, Professor J.M.Lachapelle, Professor N.Clumeck, Dr J.De Maubeuge, Dr D.Tennstedt, Dr J.André, Dr M.Bernard, Dr J.J.Stene, Dr S.De Rijcke, and Dr H.Lenain, for their help and advice during the course of this study.

References

1 Norn MS. *Demodex folliculorum*: incidence, regional distribution, pathogenicity. *Dan Med Bull* 1971; **18:** 14–17.
2 Desch C, Nutting WB. *Demodex folliculorum* (Simon) and *D. brevis* Akbulatova of man: redescription and reevaluation. *J Parasitol* 1972; **58:** 169–77.
3 Nutting WB. Hair follicle mites (Acari:Demodicidae) of man. *Int J Dermatol* 1976; **15:** 79–98.
4 Desch CE, Nutting WB. Morphology and functional anatomy of *Demodex folliculorum* (Simon) of man. *Acarologia* 1977; **19:** 422–62.
5 Du Bois. Recherche du *Demodex folliculorum* hominis dans la peau saine. *Ann Dermatol Syph* 1910; **1:** 188–90.
6 Norn MS. *Demodex folliculorum*. Incidence and possible pathogenic role in the human eyelid. *Acta Ophthalmol* 1970; Suppl. **108:** 1–85.
7 Roth AM. *Demodex folliculorum* in hair follicles of eyelid skin. *Ann Ophthalmol* 1979; **11:** 37–40.
8 Aylesworth R, Vance JC. *Demodex folliculorum* and *Demodex brevis* in cutaneous biopsies. *J Am Acad Dermatol* 1982; **7:** 583–9.
9 Andrews JRH. The prevalence of hair follicle mites in Caucasian New Zealanders. *N Z Med J* 1982; **95:** 451–3.
10 Kaufmann-Wolf M. Über regelmässiges Vorkommen von *Demodex folliculorum* in den Pusteln von Rosacea pustulosa. *Dermat Wochenschr* 1925; **81:** 1095–103.
11 Fuss F. La vie parasitaire due *Demodex folliculorum* hominis. *Ann Derm Syph (Paris)* 1933; **4:** 1053–62.
12 Beerman H, Stokes JH. Rosacea complex and *Demodex folliculorum*. *Arch Dermatol Syphilol* 1934; **29:** 874–84.
13 Breckenridge RL. Infestation of the skin with *Demodex folliculorum*. *Am J Clin Pathol* 1953; **23:** 348–52.
14 Riechers R, Kopf AW. Cutaneous infestation with *Demodex folliculorum* in man. *J Invest Dermatol* 1969; **52:** 103–6.
15 Nutting WB, Green AC. Pathogenesis associated with hair follicle mites (*Demodex* spp.) in Australian Arborigines. *Br J Dermatol* 1976; **94:** 307–12.
16 Norn MS. Incidence of *Demodex folliculorum* on skin of lids and nose. *Acta Ophthalmol* 1982; **60:** 575–83.
17 Sengbusch HG, Hauswirth JW. Prevalence of hair follicle mites, *Demodex folliculorum* and *D. brevis* (Acari: Demodicidae), in a selected human population in western New York, USA. *J Med Entomol* 1986; **23:** 384–8.
18 Ayres S Jr, Ayres S 3d. Demodectic eruptions (Demodicosis) in the human. *Arch Dermatol* 1961; **83:** 816–27.
19 De Dulanto F, Camacho-Martinez F. Demodicidose 'gravis'. *Ann Dermatol Venereol* 1979; **106:** 699–704 (Fre).
20 Ecker RI, Winkelmann RK. *Demodex* granuloma. *Arch Dermatol* 1979; **115:** 343–4.
21 Grosshans EM, Kremer M, Maleville J. *Demodex folliculorum* und die Histogenese der granulomatösen Rosacea. *Hautarzt* 1974; **25:** 166–77.
22 Purcell SM, Hayes TJ, Dixon SL. Pustular folliculitis associated with *Demodex* folliculorum. *J Am Acad Dermatol* 1986; **15:** 1159–62.
23 Miskjian HG. Demodicosis (*Demodex* infestation of the scalp). *Arch Dermatol* 1951; **63:** 282–3.
24 Seifert HW. *Demodex folliculorum* als Ursache eines solitären tuberkuloiden Granuloms. *Z Hautkr* 1977; **53:** 540–2.
25 Post CF, Juhlin E. *Demodex folliculorum* and blepharitis. *Arch Dermatol* 1963; **88:** 298–302.
26 Marks R, Dawber RPR. Skin surface biopsy: an improved technique for the examination of the horny layer. *Br J Dermatol* 1971; **84:** 117–23.
27 Hojyo Tomoka MT, Dominguez Soto L. Demodecidósis y dermatitis rosaceiforme. *Med Cutan Iber Lat Am* 1976; **2:** 83–90.
28 Bøge-Rasmussen T, Christensen JD, Gluud B *et al.* *Demodex folliculorum* hominis (Simon): incidence in a normomaterial and in patients under systemic treatment with erythromycin or glucocorticoid. *Acta Derm Venereol (Stockh)* 1982; **62:** 454–6.
29 Sato Y, Higuchi H, Saito U. Demodectic eczematoid eruption on the face of a boy receiving a long-term corticosteroid treatment. *Jpn J Dermatol* 1965; **75:** 331.
30 Siegel S, Castellan NJ. *Nonparametric Statistics for the Behavioral Sciences*, 2nd edn., Singapore: McGraw-Hill International, 1988; 137–44, 235–44.
31 Lellouch J, Lazar P. *Méthodes Statistiques en Expérimentation Biologique*, 1st edn. Paris: Flammarion, 1974; 51–2.
32 Spickett SG. Studies on *Demodex folliculorum* Simon (1842). I. Life history. *Parasitology* 1961; **51:** 181–92.
33 Marks R, Harcourt-Webster JN. Histopathology of rosacea. *Arch Dermatol* 1969; **100:** 683–91.
34 Ramelet A-A, Perroulaz G. Rosacée: étude histopathologique de 75 cas. *Ann Dermatol Vénéréol* 1988, **115:** 801–6.
35 Forton F. *Demodex* et inflammation périfolliculaire chez l'homme: revue et observation de 69 biopsies. *Ann Dermatol Venereol* 1986; **113:** 1047–58 (Fre) (Eng Abstr).
36 Rufli T, Büchner SA. T-cell subsets in acne rosacea lesions and the possible role of *Demodex folliculorum*. *Dermatologica* 1984; **169:** 1–5.

37 Grosshans E, Dungler T, Kien TT, Kremer M. *Demodex folliculorum* und Rosacea: experimentelle und immunologische Studien. *Z Hautkr* 1980; **55:** 1211–18.

38 Robinson TWE. *Demodex folliculorum* and rosacea. *Arch Dermatol* 1965; **92:** 542–4.

39 Rufli T, Mumcuoglu Y, Cajacob A, Büchner S. *Demodex folliculorum*: zur Ätiopathogenese und Therapie der Rosazea und der perioralen Dermatitis. *Dermatologica* 1981; **162:** 12–26 (Ger) (Eng Abstr).

40 Shelley WB, Shelley ED, Burmeister V. Unilateral demodectic rosacea. *J Am Acad Dermatol* 1989; **20:** 915–17.

41 Varotti C, Ghetti P, Negosanti M, Passarini B. *Demodex folliculorum* ed acne rosacea. *G Ital Dermatol Venereol* 1981; **116:** 489–91.

42 Brinnel H, Friedel J, Caputa M *et al.* Rosacea: disturbed defense against brain overheating. *Arch Dermatol Res* 1989; **281:** 66–72.

43 Grosshans E. La rosacée. *Presse Med* 1988; **17:** 2393–8 (Fre) (Eng Abstr).

44 Spickett SG. Aetiology of rosacea. *Br Med J* 1962; **i:** 1625–6. (Eng Abstr).

45 Marks R, Black MM. Perioral dermatitis: a histopathological study of 26 cases. *Br J Dermatol* 1971; **84:** 242–7.

46 Ramelet AA, Delacrétaz J. Etude histopathologique de la dermatite périorale. *Dermatologica* 1981; **163:** 361–9. (Fre) (Eng Abstr).

47 Lindmaier A, Jurecka W, Lindemayr H. Demodicosis mimicking granulomatous rosacea and transient acantholytic dermatosis (Grover's disease). *Dermatologica* 1987; **175:** 200–4.