**Mast cell distribution in normal adult skin**

A S Janssens, R Heide, J C den Hollander, P G M Mulder, B Tank, A P Oranje

**Aims:** To investigate mast cell distribution in normal adult skin to provide a reference range for comparison with mastocytosis.

**Methods:** Mast cells (MCs) were counted in uninvolved skin adjacent to basal cell carcinomas and other dermatological disorders in adults.

**Results:** There was an uneven distribution of MCs in different body sites using the anti-tryptase monoclonal antibody technique. Numbers of MCs on the trunk, upper arm, and upper leg were similar, but were significantly different from those found on the lower leg and forearm. Two distinct groups were formed—proximal and distal. There were 77.0 MCs/mm² at proximal body sites and 108.2 MCs/mm² at distal sites. Adjusted for the adjacent diagnosis and age, this difference was consistent. The numbers of MCs in uninvolved skin adjacent to basal cell carcinomas and other dermatological disorders were not different from those in the control group. Differences in the numbers of MCs between the distal and the proximal body sites must be considered when MCs are counted for a reliable diagnosis of mastocytosis. A pilot study in patients with mastocytosis underlined the variation in the numbers of MCs in mastocytosis and normal skin, but showed a considerable overlap. The observed numbers of MCs in adults cannot be extrapolated to children.

**Conclusions:** MC numbers varied significantly between proximal and distal body sites and these differences must be considered when MCs are counted for a reliable diagnosis of mastocytosis. There was a considerable overlap between the numbers of MCs in mastocytosis and normal skin.

**Abbreviations:** ATA, anti-tryptase monoclonal antibody; BCC, basal cell carcinoma; MC, mast cell; PBS, phosphate buffered saline
performed by the forearm and lower leg (unpaired Student’s t test). No differences were found between the trunk, upper arm, and upper leg, whereas the “distal” group was higher (0.929), and neither were p values for differences between the forearm and lower leg.

Skin biopsies around various dermatological disorders, and control skin (p = 0.560). The reaction was visualised using 3,3’-diaminobenzidine (Fluka Sigma-Aldrich, Zoetermeer, the Netherlands) and 30% hydrogen peroxide (Merck, Darmstadt, Germany) in PBS for seven minutes, then rinsed with tap water for two minutes. All sections were counterstained with Mayer’s haematoxylin (Fluka AG, Buchs, Germany) for 10 seconds and rinsed in running tap water for 10 minutes. Slides were immersed three times in ethanol for three minutes after incubation in PBS for five minutes. The sections were mounted in Pertex (Histolab, Göteborg, Sweden). The negative controls consisted of omitting the staining with the primary antibody.

Staining procedure

Formalin fixed, paraffin wax embedded sections (4 μm) were used. Staining was performed using mouse ATA clone AA1 (Dako, Glostrup, Denmark) as the primary antibody, as described previously. Briefly, 141 skin slides were dewaxed in xylene three times for five minutes, washed in ethanol three times for five minutes, and then washed in phosphate buffered saline (PBS; pH 7.4) three times for five minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 minutes. To block non-specific antibody binding, the slides were preincubated with protein blocking reagent (Immunologic/Labvision, Klinipath, Duiven, the Netherlands) for five minutes. The slides were incubated with primary antibody AA1, diluted 1/200 in PBS/5% bovine serum albumin for 30 minutes, rinsed in PBS/0.1% Tween twice for five minutes, and incubated with polyvalent biotinylated antibody (Immunologic/Labvision) for 10 minutes. After washing with PBS three times for five minutes, the slides were incubated with streptavidin–peroxidase (Immunologic/Labvision) for 10 minutes. The reaction was visualised using 3,3’-diaminobenzidine (Fluka Sigma-Aldrich, Zoetermeer, the Netherlands) and 30% hydrogen peroxide for 10 seconds and rinsed in running tap water for 10 minutes. Slides were immersed three times in ethanol for three minutes after incubation in PBS for five minutes. The sections were mounted in Pertex (Histolab, Göteborg, Sweden). The negative controls consisted of omitting the staining with the primary antibody.

The numbers of mast cells (MCs) were determined in three different biopsy groups, using anti-tryptase antibody staining and expressed as MCs/mm². No differences were found between perilesional skin biopsies around basal cell carcinomas (BCCs), perilesional skin around various dermatological disorders, and control skin (p = 0.560). From these three biopsy groups, the numbers of MCs were determined in five different body sites: p values for differences in MC numbers in the trunk, upper arm, and upper leg were not significant (0.929), and neither were p values for differences between the forearm and lower leg (p = 0.240). The bodies sites with similar MC numbers were placed into two groups. The “proximal” group was formed by the trunk, upper arm, and upper leg, whereas the “distal” group was formed by the forearm and lower leg (unpaired Student’s t test, p < 0.001).

MCs in all the three different biopsy groups were counted. The numbers of MCs in perilesional skin around BCCs and other skin lesions were counted and compared with those in the skin from healthy women (control group).

MC numbers, sample characteristics, and patient assessments were recorded in SPSS for Windows. Both paired and unpaired Student’s t tests were used to compare the means. A p value of < 0.05 was considered significant.

Table 1 Mean mast cell (MC) numbers in the 3 biopsy groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (SD) MC numbers</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: BCC (n = 25)</td>
<td>91.3 (36.0)</td>
<td>0.560</td>
</tr>
<tr>
<td>Group 2: other dermatological disorders (n = 95)</td>
<td>87.8 (41.8)</td>
<td>0.560</td>
</tr>
<tr>
<td>Group 3: controls (n = 21)</td>
<td>79.5 (31.8)</td>
<td>0.560</td>
</tr>
</tbody>
</table>

* p Value for differences in MCs/mm² between the three groups; Student’s t test.
RESULTS

Numbers of MCs in healthy non-lesional and perilesional skin

In sections stained with ATA, the numbers of MCs in the sections of skin from healthy women who underwent mammary reduction or abdominoplasty (control group) were compared with those in the other two biopsy groups using an unpaired parametric Student’s t test (table 1; fig 1A). The numbers of MCs in perilesional skin in BCCs (n = 25), perilesional skin from various dermatological disorders (n = 95), and skin from the healthy group (n = 21) were not significantly different from each other. The 25 BCC biopsies and 95 other dermatological disorder biopsies were obtained from five different body sites and not exclusively from the trunk. To determine the possible influence of the body site and of the adjacent diagnosis of perilesional skin on the numbers of MCs, a multiple linear regression analysis was performed. When adjusted for the body site from which the biopsies were obtained, the numbers of MCs did not depend on the adjacent original diagnosis (p = 0.992).

Table 2. Mast cell (MC) numbers according to the different body sites

<table>
<thead>
<tr>
<th>Body area</th>
<th>N</th>
<th>Mean (SD) MC/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trunk</td>
<td>17</td>
<td>78.6 (31.5)</td>
</tr>
<tr>
<td>Upper arm</td>
<td>44</td>
<td>76.5 (32.7)</td>
</tr>
<tr>
<td>Upper leg</td>
<td>29</td>
<td>74.9 (38.8)</td>
</tr>
<tr>
<td>Proximal</td>
<td>90</td>
<td>77.0 (33.6)</td>
</tr>
<tr>
<td>Forearm</td>
<td>27</td>
<td>101.2 (32.6)</td>
</tr>
<tr>
<td>Lower leg</td>
<td>24</td>
<td>113.1 (46.7)</td>
</tr>
<tr>
<td>Distal</td>
<td>51</td>
<td>108.2 (41.4)</td>
</tr>
</tbody>
</table>

Perileisional skin around basal cell carcinomas, various dermatological disorders, and the control group.

*Difference in MCs/mm² between trunk, upper arm, and upper leg: p = 0.929; **Difference of MCs/mm² between proximal and distal body areas: p = 0.900; ***Difference of MCs/mm² between forearm and lower leg: p = 0.240 (all Student’s t test).

Proximal: trunk, upper arm, and upper leg; distal: forearm and lower leg.

MCs in various body sites

Perilesional skin samples stained with ATA were used for counting MCs in biopsies from different body sites. Adjusted for the diagnosis, the numbers of MCs were significantly dependent on the body site from which the skin samples were obtained (p = 0.001) (figs 2, 3A, B).

Table 2 and fig 1B and C show the numbers of MCs at different body sites. The highest numbers of tryptase positive MCs were found in the lower leg and the forearm. These were significantly higher (p < 0.001) than was seen on the trunk, upper leg, and upper arm. As shown in table 2 and fig 2, two body site groups were formed according to this finding. The proximal group comprised biopsies obtained from the trunk, upper leg, and upper arm. The mean number of MCs in this group was 77.0 MCs/mm² (SD, 33.6). The distal group comprised biopsies obtained from the lower leg and forearm. The mean number of MCs in this group was 108.2 MCs/mm² (SD, 41.4). Adjusted for the diagnosis around which the MCs were counted (BCC or other skin lesions), a similar difference was found in the numbers of MCs at proximal and distal body sites.

The number of capillaries in a biopsy does not affect MC counts

The numbers of MCs were calculated by dividing the total number of MCs by the total area of the biopsy. When the numbers of capillaries and skin appendages were subtracted from the total area of one skin biopsy, the numbers of MCs/mm² were computed. It appeared that the numbers of capillaries and skin appendages subtracted from the total surface area had no significant influence on the accuracy of the numbers of MCs/mm². However, the number and surface area of capillaries appeared to correlate (p < 0.001) with the number of MCs (B coefficient, 1.198). When adjusted for age, there was a significant effect of the number of capillaries on the number of MCs in proximal body sites (B coefficient, 1.5; p < 0.001). The number of capillaries had no effect on the number of MCs at distal body sites (B coefficient, −0.4; p = 0.639).

Pilot study in patients with mastocytosis (comparison)

Numerous MCs are present in mastocytosis and occur as oval to spindle shaped cells with a centrally located round to oval nucleus. They are concentrated in the upper dermis and around the blood vessels (fig 3C, D).

The numbers of MCs in a pilot study group of 14 patients with mastocytosis ranged from 78 to 2409 MC/mm² (mean, 821; SD, 582). The ages of these patients ranged from 4 to 49 years, with six patients younger than 16 years. The numbers of MCs in mastocytosis were increased nearly 10 fold compared with those seen in normal skin. Figure 3E and F shows immunohistochemical staining for CD117, and an example of c-kit analysis, which is only possible in digested tissue, is shown in fig 4.
DISCUSSION

In our present study, the numbers of MCs in adults were determined in biopsies obtained from five different body sites. Higher numbers of MCs were found in the forearm and lower leg (distal extremities) compared with those in the trunk, upper leg, and upper arm (centre and proximal extremities). Mast cells on the face were not counted in our study because diagnostic biopsies for mastocytosis are preferentially not taken from the face. From a pilot study in 14 patients with mastocytosis, we concluded that the lower limit for the number of MCs in mastocytosis may be as low as 78 MCs/mm², which is also lower than the mean number of MCs in healthy skin (87.8 MCs/mm²). Thus, there was an overlap in the range of numbers of MCs in mastocytosis and normal skin.

The first reports on the measurement of MC numbers in different body sites date to 1950.5-6 In those early studies, different counting and staining techniques were used and no differences in the numbers of MCs in relation to the site of origin of the biopsy were noted. The numbers of MCs were found to be between 44 and 50 MCs/mm². The uneven distribution in the numbers of MCs in different body sites may be the result of differences in their functional, environmental, and haemodynamic properties. Although still controversial, the numbers of MCs do not appear to be related to variations in the exposure of different body sites to ultraviolet light.17-20

Collecting a large number of skin samples, using perilesional skin biopsies from BCCs or various dermatological disorders was useful, making biopsies from healthy individuals (controls) unnecessary. Cohen and Rogers emphasised the increased numbers of MCs around BCCs.22 They reported significant differences between the numbers of MCs in skin directly adjacent to a BCC compared with the surrounding skin, independent of the overall inflammatory cell response in the area. An increase in the numbers of MCs was described above and around multiple and single dermatofibromas and other benign epithelial tumours.23-24 In contrast, in other

We confirm the results obtained by Weber et al., who reported increased numbers of MCs on the face compared with other body sites and variations in numbers of MCs at different body sites similar to those reported here.21 The main difference between their study and ours is that they used the toluidine blue staining technique. It may be the case that more granules were stained in our study than that of Weber et al because the ATA technique is more sensitive than the toluidine blue stain used in their study. We also used a high magnification (×400) for analysing stained MCs from a picture on the computer screen. This may have magnified groups of granules that would have otherwise remained undetected.

“There was an overlap in the range of numbers of mast cells in mastocytosis and normal skin”

Figure 3  (A) Normal skin near basal cell carcinoma (original magnification, ×100). Mast cells occur in the normal dermis in small numbers as oval to spindle shaped cells. They are concentrated around the blood vessels. (B) Normal skin of mamma reduction tissue (original magnification, ×100). Mast cells occur in the normal dermis in small numbers as oval to spindle shaped cells. They are concentrated around the blood vessels. (C) Haematoxylin and eosin staining of mastocytosis (urticaria pigmentosa) (original magnification, ×100). Mast cells in the normal skin are indistinguishable from other perivascular cells. A small amount of granular cytoplasm is seen. (D) Urticaria pigmentosa, dense infiltrate of mast cells in the upper dermis located directly under the basal membrane. The infiltrate is also more concentrated around the blood vessels (original magnification, ×300). (E) Urticaria pigmentosa: positive staining of the mast cell infiltrate with CD117 (original magnification, ×300). (F) Urticaria pigmentosa: positive staining of the mast cell infiltrate with CD117 (original magnification, ×700).
studies there was no difference in the numbers of perilesional MCs between benign and malignant skin lesions. By using perilesional skin biopsies without obvious signs of inflammation and determining the numbers of MCs as far away from the original lesion as possible, we avoided possible influences of inflammation on MC numbers.

Although MCs are mostly seen around dermal capillaries, in our study we found that adjusting for the number of capillaries does not contribute to a more precise quantification of MCs/mm². In other studies on MC quantitation, an attempt was made to define the numbers of MCs in different dermal layers.

In our study, the dermis was not divided into different dermal layers because the border between the papillary dermis and reticular dermis was not always clearly visible, which would make counting in relation to different dermal layers less accurate.

Based on our results, it is impossible to draw a strict distinguishing line between the upper limit of MC numbers in normal skin and the lower limit of MC numbers in mastocytosis. We suggest that a figure of up to 75 MCs/mm² should be considered as normal and more than 250 MCs/mm² as abnormal. Between 75 and 250 MCs/mm² is the border zone in which a diagnosis of mastocytosis should certainly be considered. These figures are crude and an individual approach still remains essential. Further studies in a large number of patients with mastocytosis are necessary to clarify the lower limit of the numbers of MCs in these patients.

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