Cluster Analysis of Patients With Ocular Surface Disease, Blepharitis, and Dry Eye

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Objective: To develop a classification system for blepharitis and dry eye based on a classification-tree model of a large group of subjects who were given a variety of objective physiologic tests.

Methods: We evaluated 513 subjects, some healthy and some with blepharitis and dry eye, with tests for tear volume, tear flow, and tear turnover and the Schirmer test for dry eye. Meibomian gland function was evaluated by meibomian gland lipid expression for lipid volume and lipid viscosity, evaporation, and eyelid transillumination for meibomian gland drop out. We subjected these data to cluster analysis and formulated a classification tree.

Main Outcome Measure: The outcome measure of this study was the statistically valid groups of subjects with and without ocular surface symptoms identified by their physiologic characteristics.

Results: Cluster analysis most successfully grouped subjects by initially dividing them into 2 groups based on the presence or absence of gland drop out and then by lipid viscosity and volume, Schirmer test results, and evaporation. The analysis created 9 categories. This division created an objective classification system that was found to have clinical relevance. Normal subjects were distributed across several groups.

Conclusions: Using a classification tree, blepharitis and dry eye can be classified with objective physiologic tests into clinically relevant groups that have common characteristics. The analysis establishes the central role of meibomian gland dysfunction in blepharitis and demonstrates the diverse characteristics of the normal population.


We undertook a long-term project to measure a series of physiologic parameters in normal subjects and in patients with ocular surface disease including dry eye, blepharitis, and other related symptom complexes, such as rosacea and Sjögren syndrome. We performed a statistical analysis of the data to assess whether our current classification system was justified or whether we needed to search for new methods of examining patients and collecting data that could group and classify patients successfully. We hoped such an analysis would also clarify pathophysiologic mechanisms that have not been previously considered or appreciated.

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METHODS

The 513 subjects in this study were taken from our clinic population at the University of Iowa, Iowa City, and at Oregon Health and Science University, Portland. Most were referred with a diagnosis of dry eye and/or blepharitis. For each subject, we recorded an initial diagnosis of dry eye, blepharitis, rosacea, Sjögren syndrome, normal, or other. These diagnoses, except the diagnosis of normal, were not mutually exclusive. Of 662 subjects tested, 149 were eliminated for missing values, leaving 513 subjects in the final data set. The study was reviewed and approved by the institutional review board of each institution.

Patients were recruited for the normal group without reference to symptoms of dry eye or blepharitis. Thus, they were categorized as normal only to the extent that they believed themselves to be normal; they were using no eye drops or eye medications, and they had no history of eye surgery or eye disease and were not contact-lens wearers. Some of these patients may have had occasional symptoms of dry eye. A separate group of women who did not have symptoms was recruited and tested to determine mean and standard deviation values for asymptomatic subjects.

The largest single group referred for evaluation and testing came with the initial diagnosis of dry eye, and all of these subjects complained of dry eye symptoms. We did not use a standardized questionnaire to stratify the se-
The mean ± SD data for normal subjects for turnover of tear volume were reasonable and were close to those usually associated with dry eye.

We produced defining values that seemed reasonable by using the 1 standard deviation, which we designated as "other." The 2 most common diagnoses were basement membrane dystrophy (10 subjects) or ocular allergies and atopic disease (12 subjects).

The physiologic parameters tested were the same for all patients and have been previously reported. The tests for dry eye included tear osmolarity, fluorophotometric tear volume, tear flow, and tear turnover (decay constant) and the Schirmer test without anesthesia.1-3 Tear osmolarity was measured with freezing point depression using a Clifton Nanoliter Osmometer (Clifton Technical Physics, Hartford, NY). Fluorophotometry was performed with a Fluorotron Master Fluorophotometer (OcuMetrics, Mountainview, Calif). The tests for meibomian gland dysfunction and blepharitis included meibomian gland expression for lipid volume and viscosity, meibomian gland drop out, and tear film evaporation. A description of these tests has been presented in previous publications.1,4,6

Lipid volume was assessed as the average diameter of the expressible lipid measured at the slitlamp following 5 seconds of digital pressure on the lower lid. Lipid viscosity was based on a scale of 1 to 4, with 1 representing clear lipid and 4 representing very thickened, opaque viscous lipid with the consistency of toothpaste.

**CLINICAL ASSESSMENT AND CLASSIFICATION**

We assigned 1 or more physiologic diagnoses of physiologic dry eye and seborrheic or obstructive meibomian gland dysfunction, based on physiologic parameters.1,2 We also assigned a clinical diagnosis of rosacea, Sjögren syndrome, normal, or other based on the subject's initial clinical manifestation. We developed a set of rules to define physiologic dry eye based on data from a separate set of normal women. For this, a separate group of 76 normal women, aged 35 to 60 years, without any symptoms of dry eye or other eye disease, was used. We tested these subjects to determine the mean and standard deviation for 5 dry eye tests: the Schirmer test, fluorophotometric tear volume, flow and tear turnover, and tear osmolarity. A description of these tests has been presented in previous publications.1,4,6

Lipid volume was assessed as the average diameter of the expressible lipid measured at the slitlamp following 5 seconds of digital pressure on the lower lid. Lipid viscosity was based on a scale of 1 to 4, with 1 representing clear lipid and 4 representing very thickened, opaque viscous lipid with the consistency of toothpaste.

**STATISTICAL METHODS**

Hierarchical clustering (Ward minimum variance method) was initially applied to all subjects by using all physiologic variables (Schirmer test, fluorophotometric tear volume, flow and tear turnover, and tear osmolarity) and all blepharitis variables (evaporation, gland drop out, lipid viscosity, and lipid volume).1 Potential age effects in the physiologic variables were removed by using local polynomial regression before the cluster analysis.8

The results of cluster analysis were used as a starting point for a classification-tree model. This classification-tree model was fitted with 10-fold cross-validation by using all original physiologic and blepharitis variables.1 All computation was done with R statistical computing language.10

Using cluster analysis, we attempted to create groups that corresponded to clinically relevant and identifiable diagnoses. The size and properties of the data set ultimately determine the extent to which this process can be carried. We also selected for the simplest classification method that derived useful clinical information. Six clusters were initially identified from the cluster analysis. Using the 6 groups as a starting point, the final fitted classification tree divides 513 subjects into 9 categories. The Figure shows the final decision tree with decision rules and number of patients. We found we needed to use only 5 of the 13 variables to establish a classification system that corresponded, at least partially, to clinically derived systems.

These variables were, in order of their use, gland drop out, lipid viscosity, evaporation, Schirmer test value, and lipid volume. Further subdivisions did not produce additional groups that had clinical significance.

The median values of the 9 groups are presented in Table 2. Meibomian gland drop out, found only in groups 1 and 4, differentiates these 2 groups from the others. Drop out is not listed as a column in Table 2. Although the number of glands missing in each lower lid was recorded and evaluated as a variable, we found it was not necessary to use the number of glands missing. Only the presence of any gland drop out was needed to differentiate groups 1 and 4 from the others.
The median values of each variable and the statistical decision tree were then used to create a simpler decision tree, presented in Table 3. These tables show that by using only 5 clinical tests, meibomian gland drop out, lipid volume, Schirmer test value, evaporation, and lipid viscosity, subjects can be placed in their diagnostic group. The only test we used that is not routinely performed was evaporation, and this was required only to discriminate group 2a from group 2b and group 3a from group 3b. All other subjects could be categorized without testing for evaporation. Table 3 also lists a clinical description for each group that identifies its main characteristics.

We then compared the statistical classification tree with our previous clinically derived classification system that was based primarily on physiologic parameters, a dermatologic examination for rosacea, and a clinical history. The results of this comparison are presented in Table 4. Included also are the data on the presence of global ocular surface symptoms. In this comparison, each subject could have had more than 1 physiologic diagnosis, such as dry eye and obstructive meibomian gland dysfunction.

**Figure.** The final decision tree with decision rules and number of patients.

These results suggest that data can be used to classify subjects with similar characteristics that have clinical relevance and statistical validity. We believe this approach places ocular surface disease classification on a stronger scientific footing. There are several clinical implications that can be drawn from this study. The study strongly validates the fundamental division between subjects with gland drop out and those without. Meibomian gland drop out appears to be an important marker for a disease process that is relevant to this group of subjects. The study also validates the fundamental role of lipid volume since we could make statistically valid divisions between subjects with increased lipid volume and those with normal or low lipid volume. It was on the basis of lipid volume that we previously proposed differentiating patients with meibomian gland dysfunction between those with obstructive meibomian gland dysfunction (low lipid volume) and those with seborrheic meibomian gland dysfunction (high lipid volume). The cluster classification system was derived, however, through an independent and objective analysis of the data without regard to our, or other, previous classification schemes. The result is not identical to our past intuitive efforts, and we did not expect it to be. It is, however, reasonably similar.

Groups 1 and 4 contained those subjects with obstructive meibomian gland dysfunction. Group 1, which we named Rosacea, Obstructive Meibomian Gland Dysfunction, and Dry Eye, differs from group 4 by lipid volume and viscosity. Fourteen of the 19 subjects with rosacea fell into group 1, which was characterized by gland drop out and high lipid volume. Group 4 was also characterized by gland drop out but they had low lipid volume. Thus, rosacea appears to create a fairly consistent set of physiologic conditions. These 2 groups represent a primary disease process that is distinct from other forms of blepharitis. Their degree of dry eye is very high, possibly from evaporation or from corneal stimulation and secondary inhibition of the lacrimal gland. Many of these subjects might, in other systems, be classified as having staphylococcal blepharitis. It is our contention that bacteria play a role in the development of some types of meibomian gland dysfunction since Staphylococcus aureus is found somewhat more frequently in patients with blepharitis and the quantity of bacteria in the lid margin of patients with blepharitis is increased in comparison with the normal population. Every eyelid, however, is colonized by bacteria. The mere presence of these bacteria is therefore not sufficient to constitute disease. The role of bacteria on the lid margin in the development of blepharitis is most likely important but it is still not well understood. It appears, however, that blepharitis is not primarily a process of eyelid infection. It is, rather, a process that primarily involves meibomian gland function that is probably affected by bacterial action. Our study demonstrates that a meibomian gland orientation can successfully characterize a large number of subjects with blepharitis on the basis of meibomian gland function without a separate category for staphylococcal blepharitis.

Group 2 included those subjects with seborrheic meibomian gland dysfunction, with or without dry eye. Previous studies have suggested that an excess of lipid, which is a defining characteristic of this group, was responsible for their generally low evaporative rate. In the present study,
we were surprised to discover there are actually 3 separate seborrheic groups. Only 1 of these, group 2a, had the expected high lipid volume and low evaporation rate. The second group had a high lipid volume, normal viscosity, high evaporation, and dry eye. The third group appeared to have elements of both obstructive and seborrheic disease because their high lipid volume was combined with a high lipid viscosity and evaporation. These patients would be

Table 2. Medians of Physiologic and Blepharitis Variables by 9 Groups of Classification Tree

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Turnover†</th>
<th>Tear volume, µL</th>
<th>Flow, µL/min</th>
<th>Osmolarity, mmol/mL</th>
<th>Schirmer test value, mm of wetting†</th>
<th>Evaporation, $\times 10^{-7}$ g/cm² per s</th>
<th>Average lipid volume†</th>
<th>Average lipid viscosity†</th>
<th>High lipid viscosity†</th>
<th>Low lipid viscosity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>64.00</td>
<td>0.16</td>
<td>1.49</td>
<td>0.20</td>
<td>315.00</td>
<td>7.00</td>
<td>23.70</td>
<td>2.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Group 4</td>
<td>57.00</td>
<td>0.13</td>
<td>1.39</td>
<td>0.23</td>
<td>319.00</td>
<td>7.00</td>
<td>23.70</td>
<td>1.99</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Group 2a</td>
<td>39.00</td>
<td>0.19</td>
<td>2.16</td>
<td>0.49</td>
<td>300.00</td>
<td>3.00</td>
<td>2.90</td>
<td>1.00</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Group 2b</td>
<td>35.00</td>
<td>0.12</td>
<td>1.89</td>
<td>0.23</td>
<td>319.00</td>
<td>10.00</td>
<td>30.70</td>
<td>1.00</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Group 2c</td>
<td>50.00</td>
<td>0.18</td>
<td>1.62</td>
<td>0.28</td>
<td>321.00</td>
<td>12.00</td>
<td>29.00</td>
<td>1.00</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Group 3a</td>
<td>41.00</td>
<td>0.16</td>
<td>1.62</td>
<td>0.38</td>
<td>309.00</td>
<td>20.00</td>
<td>24.50</td>
<td>1.00</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Group 3b</td>
<td>51.50</td>
<td>0.16</td>
<td>1.62</td>
<td>0.38</td>
<td>311.00</td>
<td>24.50</td>
<td>24.50</td>
<td>1.00</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Group 5</td>
<td>51.50</td>
<td>0.18</td>
<td>1.62</td>
<td>0.38</td>
<td>310.00</td>
<td>24.50</td>
<td>24.50</td>
<td>1.00</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Group 6</td>
<td>54.00</td>
<td>0.18</td>
<td>1.62</td>
<td>0.38</td>
<td>314.00</td>
<td>24.50</td>
<td>24.50</td>
<td>1.00</td>
<td>0.90</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Abbreviations: DE, dry eye; MGD, meibomian gland dysfunction.

*Meibomian gland drop out, found only in groups 1 and 4, differentiates these 2 groups from the others. Drop out is not listed as a column in this table.
†Turnover refers to the percentage of turnover of tear volume per minute.
‡Performed without anesthetic.

Table 3. Final Decision Tree

<table>
<thead>
<tr>
<th>Meibomian Gland Drop Out*</th>
<th>Average Lipid Volume</th>
<th>Schirmer Test Value, mm of Wetting†</th>
<th>Average Lipid Viscosity</th>
<th>Schirmer Test Value, mm of Wetting‡</th>
<th>Cluster Analysis Group</th>
<th>Clinical Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>Normal</td>
<td>Low</td>
<td>Medium</td>
<td>Very high</td>
<td>1</td>
<td>Rosacea; obstructive MGD; DE; normal</td>
</tr>
<tr>
<td>None</td>
<td>Low</td>
<td>High</td>
<td>Normal</td>
<td>Very high</td>
<td>2a</td>
<td>Normal; seborrheic MGD</td>
</tr>
<tr>
<td>None</td>
<td>High</td>
<td>Normal</td>
<td>High</td>
<td>Very high</td>
<td>2b</td>
<td>Seborrheic MGD; DE</td>
</tr>
<tr>
<td>None</td>
<td>Normal</td>
<td>Normal</td>
<td>Low</td>
<td>Very high</td>
<td>2c</td>
<td>Normal; obstructive MGD; DE</td>
</tr>
<tr>
<td>None</td>
<td>Normal</td>
<td>Low</td>
<td>High</td>
<td>Very high</td>
<td>3a</td>
<td>Normal; low evaporation; DE</td>
</tr>
<tr>
<td>None</td>
<td>Normal</td>
<td>High</td>
<td>High</td>
<td>Very high</td>
<td>3b</td>
<td>High Schirmer test value; high evaporation; normal</td>
</tr>
<tr>
<td>None</td>
<td>Normal</td>
<td>Low</td>
<td>High</td>
<td>Very high</td>
<td>3c</td>
<td>Low flow; high evaporation; DE</td>
</tr>
</tbody>
</table>

Abbreviations: DE, dry eye; MGD, meibomian gland dysfunction.

*Drop out refers to meibomian gland drop out in lower eyelid.

Table 4. Cross-Tabulation Between the Groups From the Cluster Classification and the Physiologic Diagnosis*

<table>
<thead>
<tr>
<th>Cluster Group</th>
<th>Subjects</th>
<th>Symptomatic Subjects</th>
<th>Normal Subjects†</th>
<th>Physiologic DE</th>
<th>Obstructive MGD</th>
<th>Seborrheic MGD</th>
<th>Rosacea</th>
<th>Sjögren Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1‡</td>
<td>151</td>
<td>122</td>
<td>19 (12)</td>
<td>105</td>
<td>151</td>
<td>31</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>4‡</td>
<td>37</td>
<td>33</td>
<td>1 (3)</td>
<td>29</td>
<td>36</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2a</td>
<td>21</td>
<td>8</td>
<td>12 (57)</td>
<td>12</td>
<td>1</td>
<td>21</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2b</td>
<td>9</td>
<td>3</td>
<td>5 (55)</td>
<td>5</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2c</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3a</td>
<td>105</td>
<td>61</td>
<td>27 (26)</td>
<td>80</td>
<td>2</td>
<td>17</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3b</td>
<td>12</td>
<td>8</td>
<td>4 (33)</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>101</td>
<td>77</td>
<td>16 (16)</td>
<td>75</td>
<td>27</td>
<td>14</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>42</td>
<td>25 (36)</td>
<td>56</td>
<td>5</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>513</td>
<td>361</td>
<td>109</td>
<td>368</td>
<td>225</td>
<td>111</td>
<td>19</td>
<td>17</td>
</tr>
</tbody>
</table>

Abbreviations: DE, dry eye; MGD, meibomian gland dysfunction.

*Values are expressed as number of subjects unless otherwise indicated.
†Values are expressed as number (percentage) of normal subjects in group.
‡Meibomian gland drop out, found only in groups 1 and 4, differentiates these 2 groups from the others. Drop out is not listed as a column in this table.
classified as mixed by many other clinically based classification systems.

Dry eye without meibomian gland dysfunction is also a distinct entity, and these subjects were clustered in group 5. Half of those with Sjogren syndrome were found in this group. The other groups were mostly variations of normal, some with high evaporation and some with low evaporation. The analysis illustrates the difficulty that all classification systems have with the wide spectrum of findings in normal individuals. There are several reasons for this. Blepharitis and dry eye symptoms are very common in the general population, and some elements of meibomian gland dysfunction are frequently found in the population that considers itself to be normal. More than half of groups 2a and 2b were normal subjects, and one third of groups 3b and 6 were normal subjects as well. Analyzed differently, 25% of the normal subjects were allocated into group 3a and 23% were allocated into group 6 while 17% were allocated into group 1. Even the population of symptom-free women demonstrated a fairly wide range of variability in their physiologic measurements that overlapped disease states. This is a good indication that symptoms are not tightly linked to these physiologic measurements. Therefore, it is not surprising that we found a few normal subjects in many of these groups. Our data describing the asymptomatic, normal population were based only on women, and this represents a potential limitation of our study. This was a sample of convenience based on the much greater availability of women for study purposes.

This analysis uncovered data that may shed some light on groups to be found within the normal population. We found 2 groups with a relatively high percentage of normal subjects with high evaporation (groups 3b and 6), while other normal subjects (part of group 3a) had low evaporation. Thus, there appear to be 2 distinct types of normal subjects, 1 with high evaporation and 1 with low. This may be explained by lipid composition. There is a reasonable likelihood that evaporation is partly controlled by components of the lipid layer. One component of particular interest is cholesterol ester, because Shine and McCulley, and Shine et al found a basic division in the general population between those with low levels of cholesterol ester in their meibomian gland secretions and those with high levels. This is the only consistent division they have found in the normal population.

We found evaporation was a key variable in differentiating some clusters. This suggests that evaporation is a key process and that it plays an important role in ocular surface disease. Evaporation is not intrinsically difficult to measure but it requires a practiced technician and specialized equipment that is not generally available. Few physicians, therefore, have much experience with this fundamental process, and its use has been limited to research. Although we found no real substitute for its measurement at the present time, without evaporation, subjects can still be usefully classified up to this final point. There are, however, fundamental differences between groups that only evaporation can evaluate at present.

Evaporation may not be of critical importance in determining the care and therapy that patients need. This cluster analysis does not necessarily translate directly into a therapeutic algorithm and is not presented as such. The current analysis is exploratory by nature and should instead provide insights into ocular surface disease processes that lie behind the symptoms and the subjective clinical manifestations that we usually use to identify and treat dry eye, blepharitis, and ocular surface disease. Physiologic parameters most likely permit clustering for reasons that are based on pathophysiologic mechanisms, although these remain poorly understood. A more complete explanation of gland drop out, lipid viscosity, seborrhea, and high evaporation will lead to a better understanding of ocular surface disease and more directed and effective treatments. The use of simple and objective measurements to classify subjects also aids this process because it permits the valid identification and comparison of treatments between different clinical centers. This has been a major problem in dry eye and ocular surface disease research.

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REFERENCES