Assessment of disease activity in Graves' ophthalmopathy

Gerding, M.N.

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Introduction
"ASSESSMENT OF DISEASE ACTIVITY IN GRAVES' OPHTHALMOPATHY".

Contents

1.1 Introduction
history of the disease
epidemiology
pathology
clinical symptomatology and classification of GO

1.2 Concept of disease activity
natural history
histology
therapeutical implications

1.3 Assessment of disease activity
clinical assessment
• observation over time
• clinical activity score (CAS)
imaging techniques
• magnetic resonance imaging (MRI)
• ¹¹¹In-DTPA-Octreotide scintigraphy
• ultrasonography
laboratory measurements
• cytokines
• adhesion molecules
• TSH-Receptor antibodies
• glycosaminoglycans (GAGs)

1.4 Outline of the present thesis
Chapter 1

1. Introduction

History
It was in 1786 when the Welsh physician C. Parry suggested that the hyperthyroid state, heart failure and goitre were related. Parry described protrusion of the eyes (exophthalmos) in his first case, but his observations were not published until 1825, three years after his death. In 1835 Robert Graves (1796-1853) described three patients with palpitations, and enlargement of the thyroid gland. One case also had exophthalmos. The first author to name this disorder Graves’ disease was Trousseau in 1860. Baron C.A. von Basedow wrote about four patients with exophthalmos, goitre and palpitation in 1840. He was also the first to describe a brawny swelling of the legs (now known as "pretibial myxoedema", which occurs in a small proportion of patients with thyroid eye disease). The name Graves’ disease prevails nowadays, and the orbital disorder is now often referred to as Graves’ ophthalmopathy (GO).

Epidemiology
Graves’ ophthalmopathy usually occurs in patients presenting with, or known to have Graves’ hyperthyroidism, although in approximately 20% no thyroid dysfunction is clinically apparent. This latter situation is referred to as ‘euthyroid Graves’ disease’. However, in half of these patients evidence can be found for the existence of autoimmune thyroid disease (TPO antibodies, TSH-receptor antibodies, abnormal thyroid stimulation and/or suppression tests). Among patients with Graves’ ophthalmopathy and hyperthyroidism, the eye disease occurs after or concomitantly with the onset of hyperthyroidism, whereas in the remaining patients the thyroid disease starts after the onset of the ophthalmopathy. Thus, the majority of patients with so-called euthyroid or ophthalmic Graves’ disease will develop hyperthyroidism later in the course of the disease. In approximately 5% of the ophthalmopathy patients hypothyroidism is diagnosed.
Conversely, 30-45% of patients with established Graves' hyperthyroidism have discrete eye signs or symptoms, whereas clinically manifest ophthalmopathy is seen in 10-25%. The remaining patients without eye complaints do have, however, mostly subtle, subclinical orbital involvement as evident from eye muscle enlargement on CT scan or MRI. Thus, in almost all patients with ophthalmopathy there is evidence for autoimmune thyroid disease, and in almost all Graves' hyperthyroidism patients some signs of ophthalmopathy can be found. It is therefore our opinion, that both are manifestations of one multi-organ disorder: Graves' disease.

The prevalence of Graves' ophthalmopathy is not well established. An estimate of its incidence can be derived from two studies (Table 1.1). In 1977, a population survey was performed in 2,779 inhabitants of Wickham County, UK. These subjects lived in an iodine replete area, and the population closely resembled the English population as a whole in terms of age, sex, and social class. In 2.7% of the females and in 0.23% of the males, thyrotoxicosis (regardless of its cause) was diagnosed. A follow-up survey was done 20 years later in the original cohort and the prevalence of thyrotoxicosis had risen to 3.9% in women, whereas no new cases were observed in men. From this observation, the annual incidence of thyrotoxicosis was 0.8/1,000 women/year. From a large European study we know that approximately 60% of the cases of thyrotoxicosis are caused by Graves' disease (the contribution of Graves' disease is higher in iodine replete areas than in iodine deplete areas), resulting in a conservative estimation of an annual incidence of Graves' hyperthyroidism in women of 0.5% in the Netherlands. We know that 30-45% of the patients with Graves' hyperthyroidism have some signs or symptoms of ophthalmopathy, thus the annual incidence of Graves' eye disease would be 0.15-0.23%. Using the data from the Dutch Statistics Bureau (http://www.cbs.nl), the adult female population of the Netherlands is 5,976,000. The yearly incidence of Graves' ophthalmopathy would thus be: 896-1374 female patients. Assuming that only 10-25% of the patients with Graves' thyroid disease have clinically significant ophthalmopathy (estimated annual incidence for GO:
0.05-0.13 °/oo), 299-777 new ophthalmopathy patients would be seen in The Netherlands.

**Table 1.1.A** Estimation of the incidence of Graves’ ophthalmopathy (GO) in the Netherlands, calculated from data of the Whickham survey in the United Kingdom.

<table>
<thead>
<tr>
<th></th>
<th>adult females</th>
<th>adult males</th>
<th>No. Dutch females</th>
</tr>
</thead>
<tbody>
<tr>
<td>prevalence thyrotoxicosis</td>
<td>Whickham '77</td>
<td>2.7 %</td>
<td>0.23 %</td>
</tr>
<tr>
<td>prevalence thyrotoxicosis</td>
<td>Whickham '97</td>
<td>3.9 %</td>
<td>0.23 %</td>
</tr>
<tr>
<td>annual incidence thyrotoxicosis Whickham '97</td>
<td>0.8 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>estimated annual incidence GH (60% of thyrotoxicosis)</td>
<td>0.5 °/oo</td>
<td>2988</td>
<td></td>
</tr>
<tr>
<td>estimated annual incidence discrete GO (30-45 % from GH)</td>
<td>0.15 - 0.23 %</td>
<td>896 - 1374</td>
<td></td>
</tr>
<tr>
<td>estimated annual incidence overt GO (10-25 % from GH)</td>
<td>0.05 - 0.13 %</td>
<td>299 - 777</td>
<td></td>
</tr>
</tbody>
</table>

The second study was performed by the Mayo Clinics. Using their well kept records, the Mayo Clinic identified 120 Graves’ ophthalmopathy patients residing in Olmstead County. Since probably all patients from Olmstead County, MN, will visit Mayo, and because the demography of this county is exactly known, they could calculate an age-specific incidence rate of ophthalmopathy (Table 1.1.B). Using the Dutch age-specific population data, the annual incidence of ophthalmopathy in the Netherlands should be approximately 1200 females. However, this estimate for The Netherlands should be handled with caution. First, there is difference in iodine intake between Olmstead County (high iodine intake) and The Netherlands (borderline sufficient), and secondly it is unknown how important the ophthalmopathy was in the index cases from Minnesota. For
instance, in only 74% an ophthalmic assessment was done, suggesting that minor cases were also included.

**Table 1.1.B** Estimation of the age adjusted number of female patients with Graves' ophthalmopathy in the Netherlands (NL), calculated from the data of Olmsted County, USA (age adjusted incidence rates per 100,000 population per year).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>age-adjusted incidence rate in females in Olmsted County (n/100,000/yr)</th>
<th>no. of females in Dutch population (n/100,000) (1998)*</th>
<th>estimation of age-adjusted incidence (absolute no.) in females in NL (1998)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-24</td>
<td>10,3082</td>
<td>4,869</td>
<td>50</td>
</tr>
<tr>
<td>25-29</td>
<td>15,3820</td>
<td>6,249</td>
<td>96</td>
</tr>
<tr>
<td>30-34</td>
<td>19,5207</td>
<td>6,422</td>
<td>125</td>
</tr>
<tr>
<td>35-39</td>
<td>12,9116</td>
<td>6,273</td>
<td>81</td>
</tr>
<tr>
<td>40-44</td>
<td>32,8299</td>
<td>5,808</td>
<td>191</td>
</tr>
<tr>
<td>45-49</td>
<td>18,8192</td>
<td>5,609</td>
<td>106</td>
</tr>
<tr>
<td>50-54</td>
<td>24,5602</td>
<td>5,228</td>
<td>128</td>
</tr>
<tr>
<td>55-59</td>
<td>27,8658</td>
<td>3,983</td>
<td>111</td>
</tr>
<tr>
<td>60-64</td>
<td>35,8723</td>
<td>3,536</td>
<td>127</td>
</tr>
<tr>
<td>65-69</td>
<td>13,1729</td>
<td>3,405</td>
<td>45</td>
</tr>
<tr>
<td>70-74</td>
<td>14,6987</td>
<td>3,109</td>
<td>46</td>
</tr>
<tr>
<td>75-79</td>
<td>11,5788</td>
<td>2,593</td>
<td>30</td>
</tr>
<tr>
<td>&gt;80</td>
<td>19,0687</td>
<td>3,446</td>
<td>66</td>
</tr>
<tr>
<td>totals</td>
<td></td>
<td></td>
<td>1202</td>
</tr>
</tbody>
</table>

From these estimates, Graves' ophthalmopathy appears not to be a very rare disorder. However, it is likely that the majority of these patients will have minor, transient thyroid-associated eye changes. The incidence of more severe ophthalmopathy, e.g. disfiguring proptosis, diplopia, sight loss, as dealt with in this thesis, is probably much lower. The Academic Medical Center is one of the national referral centers for this disease, and we see approximately 100 new cases per year. From all these data, we think that a fair estimate of the yearly incidence of significant ophthalmopathy is ~400 patients.

Graves disease clusters in families, but the inheritance is polygenetic and the most important genes involved are not yet known. There is a female preponderance, with a male:female ratio of 1:3, somewhat lower than the ratio in Graves' hyperthyroidism (1:5). It is likely that a number of environmental factors are involved in the occurrence of Graves' disease. In the case of Graves' ophthalmopathy, smoking is a strong environmental risk factor for more severe eye disease, and the eye disease is often more severe in males, in old age, and in diabetes.

Pathology in Graves' ophthalmopathy

The most prominent finding is swelling of the extraocular eye muscles (EOM) at macroscopic examination, together with an increase in orbital connective- and fat tissue. Rundle et al. weighted the extra-ocular muscles of a 69 years old male with severe GO since 7 months, who died from a myocardial infarction. His EOM had all swollen to 3-to 5-fold, including the levator palpebrae (Table 1.2). The swollen muscles felt rubber-like and some had a diameter of more than 1 cm. On microscopy the muscles showed fibrosis, edema, and lymphocytic infiltration. The swelling of the muscles was not due to an increase in the number of fibers, but to an increase in the volume of the individual fibers plus an increase in connective tissue. The water content was increased, but in this case the authors did not observe an increase in fatty tissue weight. In their earlier series in patients with
GO who died in the thyrotoxic state, they did find such an increase in adipose tissue.\textsuperscript{16}

\textbf{Table 1.2} \textit{Weight of the extraocular eye muscles (mean of right and left orbit) in a 63 yrs old male with malignant GO of recent onset, who died from a myocardial infarction. Weights are compared to the mean weights found in 29 controls.}\textsuperscript{15}

<table>
<thead>
<tr>
<th>Muscles</th>
<th>weight (g)</th>
<th>mean ±SD weight in 29 controls</th>
<th>Fold increase in weight vs controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levator palpebrae</td>
<td>1.46</td>
<td>0.41±0.06</td>
<td>3.7</td>
</tr>
<tr>
<td>Lateral rectus</td>
<td>2.17</td>
<td>0.72±0.10</td>
<td>3.3</td>
</tr>
<tr>
<td>Superior rectus</td>
<td>2.20</td>
<td>0.49±0.06</td>
<td>4.9</td>
</tr>
<tr>
<td>Inferior rectus</td>
<td>2.86</td>
<td>0.66±0.08</td>
<td>4.2</td>
</tr>
<tr>
<td>Medial rectus</td>
<td>3.41</td>
<td>0.87±0.11</td>
<td>3.8</td>
</tr>
</tbody>
</table>

There is no consensus as to whether the adipose tissue compartment is swollen in GO. Both Feldon et al.\textsuperscript{17}, and Trokel et al.\textsuperscript{18} could not detect a significant increase in adipose tissue volume on CT-scan. On the other hand using three-dimensional volumetric CT-scans, van der Gaag et al.\textsuperscript{19} found swelling of the EOM alone in 20\% of their 40 GO patients; 48\% both the EOM and the adipose tissues were swollen, in 28\% the EOM volume was normal but the adipose tissue compartment had increased in volume, and in 4\% no increase in orbital tissues was apparent. Using a similar technique, Forbes et al.\textsuperscript{20} reached similar figures. It therefore appears that muscles, fat as well as the lacrimal gland, all can increase considerably in volume.

Already in the earliest descriptions it was noted that the swelling of retrobulbar tissues is due to an increase in what was called ground-substance. Now we know that this consists of collagen and glycosaminoglycans (GAG), which can be found throughout the muscle fibers in the endomysial space.\textsuperscript{21} The GAG most
prominently present is hyaluronan. Despite this accumulation of GAG's and collagen between the muscle fibers, there is no evidence for ultrastructural damage to the EOM cells themselves. The sarcomeric organization remains intact, and electron microscopy has not revealed any subcellular damage. In addition to these depositions, many authors have found an increased number of fibroblasts within the endomyseal space and the connective/adipose tissue. These infiltrating fibroblasts are considered to be the cells responsible for the overproduction of GAG's. GAG's are hydrophilic and can thus attract water and cause edema of the eye muscles and other affected retrobulbar tissues. Apart from these fibroblasts, there is also a mononuclear cell infiltration, consisting of lymphocytes, macrophages, and plasma cells. The lymphocytes are predominantly T-cells, but a few B-cells are also present. Further immunohistochemical analysis reveals a predominance of CD3+ T-lymphocytes, consisting of helper CD4+ T-cells, and a variable predominance of suppressor/cytotoxic T cells (CD8+). Molecular analysis of the T cell antigen repertoire showed a marked degree of restriction of T cell variable region gene families, suggesting an oligoclonal immune response directed against a limited number of antigenic epitopes in the orbital tissues.

Besides an increase in the volume of all retrobulbar tissues, the retrobulbar pressure appears also to be increased. Otto validated an intraorbitally positioned micro-pressure transducer to measure the retrobulbar pressure in monkeys. He used this method in GO patients who were decompressed surgically. In eight orbits with 'malignant' ophthalmopathy (e.g. sight loss due to optic nerve involvement), the retrobulbar pressure ranged from 17-40 mmHg (mean: 29 mmHg), and fell upon decompression by 9-12 mmHg. In contrast in two patients decompressed because of rehabilitative reasons, the retrobulbar pressure was only 9 and 11 mmHg, and did not change after the decompression, which was successful in terms of proptosis reduction.
**Clinical symptomatology and classification of GO**

The clinical manifestations of GO can be explained in a mechanistic sense from the increase in size of retro-ocular tissues, and the increase in retrobulbar pressure. The main clinical features of GO can be described using the NOSPECS classification (Table 1.3).\textsuperscript{31,32}

**Tabel 1.3** Modified NO SPECS classification of eye changes in Graves' disease.\textsuperscript{31,32,48,49}

<table>
<thead>
<tr>
<th>Class</th>
<th>Grade</th>
<th>Suggestions for grading</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>No physical signs or symptoms</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Only signs</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td><strong>Soft tissue involvement</strong></td>
</tr>
<tr>
<td>0</td>
<td>a</td>
<td>absent</td>
</tr>
<tr>
<td>0</td>
<td>b</td>
<td>minimal</td>
</tr>
<tr>
<td>0</td>
<td>c</td>
<td>moderate</td>
</tr>
<tr>
<td>0</td>
<td>c</td>
<td>marked</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Proptosis 3 mm or more above upper normal limit; grading for Caucasian race</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>absent</td>
</tr>
<tr>
<td>0</td>
<td>a</td>
<td>23-24 mm</td>
</tr>
<tr>
<td>0</td>
<td>b</td>
<td>25-27 mm</td>
</tr>
<tr>
<td>0</td>
<td>c</td>
<td>≥28 mm</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td><strong>Extraocular muscle involvement:</strong></td>
</tr>
<tr>
<td>0</td>
<td>a</td>
<td>limitation of motion at extremes of gaze</td>
</tr>
<tr>
<td>0</td>
<td>b</td>
<td>evident restriction of motion</td>
</tr>
<tr>
<td>0</td>
<td>c</td>
<td>fixation of a globe or globes</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td><strong>Corneal Involvement</strong></td>
</tr>
<tr>
<td>0</td>
<td>a</td>
<td>stippling of cornea</td>
</tr>
<tr>
<td>0</td>
<td>b</td>
<td>ulceration</td>
</tr>
<tr>
<td>0</td>
<td>c</td>
<td>clouding, necrosis, perforation</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td><strong>Sight loss due to optic nerve involvement</strong></td>
</tr>
<tr>
<td>0</td>
<td>a</td>
<td>absent, vision ≥0.8</td>
</tr>
<tr>
<td>0</td>
<td>b</td>
<td>Disc pallor, visual field defect, vision 0.63-0.5</td>
</tr>
<tr>
<td>0</td>
<td>c</td>
<td>Same, but vision 0.4-0.1</td>
</tr>
<tr>
<td>0</td>
<td>c</td>
<td>Same, but vision &lt;0.1-blindness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grading according to diplopia:</td>
</tr>
<tr>
<td>0</td>
<td>a</td>
<td>intermittent (when fatigued)</td>
</tr>
<tr>
<td>0</td>
<td>b</td>
<td>inconstant (present but not in primary gaze)</td>
</tr>
<tr>
<td>0</td>
<td>c</td>
<td>constant (present in primary gaze)</td>
</tr>
</tbody>
</table>
**Chapter 1**

**Class 1. Only signs, no symptoms.** This refers to the upper eyelid retraction frequently observed in patients with Graves' thyroid disease. This retraction causes stare, and lid lag on downward gaze (Von Graefe's sign) and can be due to swelling of the levator muscle. However, thyrotoxicosis per se can also induce this sign by increasing the sympathetic tone, and Von Graefe's sign is sometimes also present in hyperthyroidism not caused by Graves' disease. Sympathetic overactivity is not the only cause of lid retraction, because overactivity of the intraocular muscles (causing slower accommodation due to an increase in sympathetic tone) was shown to be unrelated to the presence or absence of upper eyelid retraction. Therefore, it is likely that eyelid retraction is multifactorial in origin and it might well be that adhesions around the levator muscle are a cause of retraction. This would explain why upper eyelid retraction frequently remains present when GO patients are rendered euthyroid.

**Class 2. Soft tissue involvement.** This entails chemosis (edema of the conjunctiva), conjunctival injection and redness, and swollen upper and lower eyelids. These findings are partly explained by an impaired venous drainage as a result of the increase in retrobulbar tissue volume. This cannot be the only reason, because retrobulbar pressure might not always be elevated, as indicated by a normal removal rate of retro-bulbarly injected labeled fluid, and soft tissue swelling does not always disappear completely after decompressive surgery. Another explanation for the swelling of the eyelids is herniation of retrobulbar tissue through the naturally occurring herniations in the orbital septum.

**Class 3. Proptosis.** Because of the bony confinement of the orbit, the swollen retrobulbar tissue has no other outlet than pushing the globe forward. Hence, proptosis has even been termed "nature's own decompression". In their meticulous postmortem studies Rundle and Pochin demonstrated that the normal orbital volume of the eye muscles is about 3.5 ml and that of the orbital cavity 26 ml. They then showed that an increase in muscle volume of 4 ml causes a
proptosis of 6 mm. Thus, small changes in tissue volume can cause considerable proptosis. In the NO SPECS system, proptosis is heavily underestimated since Hertel values of 21-22 mm in a Caucasian patient are not scored, although it may be very relevant to the patient. In the Netherlands the proptosis in healthy females ranges from 9-16 mm (mean value 12.3 mm) and in healthy males from 8-18 mm (mean 13.5 mm). This sex difference in 160 eyes was significant. The Pearson correlation coefficient for the interobserver variation in measuring exophthalmos was 0.88.

Class 4. Extraocular muscle involvement. One can easily imagine that swelling of the EOM leads to impaired mobility. If impairment is asymmetrical, the patient will have double vision. It is important to realize that the diplopia is of a binocular nature: if impairment is exactly symmetrical, or has become so by adapting the head position (ocular torticollis), there will be no diplopia. The same might occur, if the visual acuity in one eye is low.

For a long time, the diplopia was ascribed to paresis of one or more of the EOM, hence the term "ophthalmoplegia". However, we now know that the mobility impairment is caused by restricted relaxation of the affected antagonist. This can be appreciated by the "forced duction test". By actually grasping the globe and attempting to move it in the affected direction, mechanical resistance is felt.

Class 5. Corneal involvement. Exophthalmos, lid retraction, and less frequent blinking all contribute to overexposure of the cornea, which can lead to keratitis. Early signs are photophobia, a gritty sensation, blurred vision, and intolerance to contact lenses. Corneal irritation is easily relieved and prevented by the liberal application of artificial tears, eye ointments, dark shields etc.

Class 6. Sight loss. The development of exophthalmos diminishes the retrobulbar pressure. Sometimes this mechanism ("nature's own decompression") fails and
this may eventually lead to optic nerve damage. This is in agreement with the fact that the presence of optic neuropathy is unrelated to the degree of proptosis.\textsuperscript{42} It has often been noticed that the patients with optic nerve involvement have relatively low proptosis readings.\textsuperscript{38,43} Koornneef suggested that a well developed, tight orbital septum might preclude proptosis and nature’s decompression, resulting in higher retrobulbar pressures and optic neuropathy.\textsuperscript{30,38}

Sight loss due to optic nerve damage can be accompanied by visual field defects and impaired color vision.\textsuperscript{44} There is no evidence for a direct inflammation of the optic nerve itself, and optic neuropathy is probably due to swelling of the EOM close to the apex of the orbit: this can be seen on coronal CT-or MRI scan as "apical crowding", which is a risk factor for optic neuropathy.\textsuperscript{45} The enlarged muscles might very well cause neuropathy by direct pressure on the nerve or its blood supply.\textsuperscript{42} This latter possibility is supported by the observation of swelling of the nerve sheath suggesting venous stasis.\textsuperscript{18}

\textit{Assessment of severity of Graves’ ophthalmopathy}

Originally the NO SPECS system was designed to describe the variety of clinical features of GO, serving as a memory aid for the examination of patients. However, it has later been used in the follow-up of patients undergoing treatment for their eye disease. This latter use of the NO SPECS has been criticized by many authors.\textsuperscript{46-48} Firstly, because the grading of most classes is subjective. Secondly, because grading in some classes is inappropriate; e.g. class 4.c is virtually never seen, diplopia is not assessed, and a proptosis of 22 mm is not scored. Thirdly, the classes suggest an hierarchy which does not exist; e.g. class 5 can be present in patients with lid retraction only. These criticisms have led to recommendations by an International Committee, published in 1991,\textsuperscript{49} advocating the use of objective, reliable and validated measurements of those eye changes which are relevant to the patient. How can this be achieved?

\textit{Soft tissue involvement.} Measurements are possible of the lid aperture, and of the position of the upper and lower eye lid assessed as upper and lower scleral show
respectively in mm. The inter- and intra-observer variability of these measurements are not previously known. An important aspect of soft tissue involvement is the swelling of the eye lids, which can be disfiguring. There are no widely accepted ways to measure this swelling, and the current assessment is subjective. We use colour slides, taken in two directions (‘en face’ and ‘en profil’), to improve the assessment of eye lid swelling. Unfortunately, the inter-observer agreement of assessing the severity of eye lid swelling is disappointingly low. The colour slides, however, are superior to the traditional grading of soft tissue involvement at each visit in assessing the response to immunosuppression, because slides can be compared directly.

Proptosis. The measurement is quantitative and rather reliable. The range of normal values is, however, rather wide. Whether e.g. 18 mm of exophthalmos is abnormal will depend on the degree of proptosis prior to the onset of the eye disease.

Eye muscle involvement. EOM motility can be measured quantitatively using different techniques. Mourits et al have developed a reliable and reproducible technique using a modified hand perimeter. For the patient, diplopia is much more relevant than restriction of EOM motion. It is hard to quantify diplopia, but Bahn and Gorman have proposed an useful grading: 0. No diplopia, A. Intermittent diplopia (present only when fatigued), B. Inconstant diplopia (only in certain directions of gaze), C. Constant diplopia (in straight gaze, correctable with prisms), D. Constant diplopia (uncorrectable with prisms).

Corneal involvement. Its use in evaluating the severity of the disease is restricted by the almost routinely described artificial tears to GO patients, which are effective in treating and preventing corneal changes.

Sight loss. Visual acuity is to a certain degree subjective, but easily quantifiable.

In addition, the Committee recommended to include an assessment of disease activity (see next section), and a patient self assessment score in the evaluation of treatment results. Indeed, the quality of life is markedly decreased in patients with
GO.52 We developed a disease specific Quality-of-Life questionnaire, that appears to be a reliable instrument.53

These outcome measures are especially valuable to evaluate a treatment aimed at improving one aspect of the disease: decrease in proptosis readings after decompression, increase in eye muscle motility and improvement of diplopia upon squint surgery, improved Quality-of-Life after blepharoplasty. However, many authors feel the need for an overall assessment of the severity of the ophthalmopathy. Such an integrated score, or index can be used to relate severity to the level of antibodies, smoking habits, and other possible determinants. It might also be useful to analyze a treatment aimed at different aspects, like immunosuppressive therapies which might improve soft tissue swelling, proptosis, diplopia etc. For this purpose, the Ophthalmopathy Index (OI) has been used extensively.54-57 However, the OI gives equal weight to all features, whereas sight loss and diplopia might be more important to the patient than proptosis and eye lid swelling. To include a weighing factor, the Total Eye Score (TES) was developed.58-59 The TES is calculated by multiplying each class of the NO SPECS system (except class 5) by its grade of severity (0=0, a=1, b=2, c=3 points), yielding a maximum total score of 45 points.

Thus, a therapy can be evaluated by the changes in the different features. However, we know that the medical therapies are not always successful, and there is a need to determine how many patients respond to a therapy, and how many are non-responders. Many different response criteria have been used in the past, but in view of the above mentioned Recommendations new criteria have been established by Bartalena et al.60 In the studies described in this thesis we have used similar criteria, all based on quantitative (where possible) and objective assessments of the various features of the eye disease. The response criteria are:

**Major criteria:**
1) changes in diplopia of ≥1 grades on the diplopia scale.
2) changes in visual acuity of ≥1 lines on the Snellen chart
3) changes of ≥8 degrees in eye muscle motility (elevation)

**Minor criteria:**
1) changes of ≥2 mm in proptosis
2) changes of ≥2 mm in lid aperture
3) changes in soft-tissue involvement, assessed from colour slides;

Patients who improved in at least in one major, or in ≥2 minor criteria are considered responders. Patients in whom no changes occurred, or who responded in only one minor criterion, or who actually deteriorated are classified as non-responders.

An important reason to differentiate between responders and non-responders is the hypothesis that patients with active disease respond to immunosuppression, whereas inactive patients will not. This concept will be explained in the following section.

1.2 Concept of disease activity
The concept of disease activity originates from observations of the natural course of the eye signs in patients who were not treated for the eye disease, and from a small number of histologic studies performed on orbital tissues from patients with variable duration of the eye disease.

Observations on the natural history of Graves’ ophthalmopathy.

In 1923 ophthalmopathy patients were treated by "skillful neglect" and by "sending the patients to the country". Nevertheless, Kessel et al noted that there was a tendency towards spontaneous regression of the eye signs over time, though without reaching the pre-morbid state. These patients however were also left untreated for their hyperthyroidism, (which improved spontaneously as well), and no specific eye measurements were reported. Copper was one of the first authors to do specific measurements. He showed that the retrobulbar pressure, measured by an orbitonometer, decreased over time. It was Rundle who did sequential measurements in 12 patients who were not treated for the ophthalmopathy. He followed these patients during 2½ years, recorded proptosis readings and measured the impairment of elevation using a vertometer. He found that the eye disease
begins with a dynamic phase, characterized by exacerbations and remissions, followed by a static phase: as depicted in Rundle's curve in Figure 1.1. A similar curve was made by Dobyns. Both authors agreed that despite some remission, the eye disease often is still severe when the static phase is reached.

Figure 1.1 Rundle's curves, describing the natural course of proptosis, eye muscle motility, and upper lid margin relative to the limbus over time.

Later studies have confirmed these early observations. Perros et al., found that spontaneous improvement occurred in 64% of his 59 patients during a median follow-up of 12 months, while the disease remained stable in 22% and progressed in 14%. Despite this high incidence of improvement, it is also clear that most patients think that their eyes never returned to normal: 54% (out of 120) in the
study by Hamilton et al.,\textsuperscript{67} and 61\% (out of 120) in a study of Bartley et al., with a median follow-up of 9\textfrac{1}{2} years.\textsuperscript{68}

Thus, most authors agree that the orbital disease starts with a period in which the disease gets worse: the active phase, in which there can be exacerbations and remissions up to a plateau is reached in which the disease is at its most severe stage. This plateau phase is then followed by a slow decrease in severity until a stable end-stage is reached: the inactive phase. However, often the remaining eye signs and symptoms are still invalidating the patient.

Rundle's curve is generally accepted, despite the fact that the time axis is not clearly defined (figure 1.1): It is unclear how long it takes in individual patients before the stable end-stage is reached. This can take between several months to 5 years.\textsuperscript{3} Even less is known about the pathogenesis of the transition from active into inactive disease. Here we will review the available histologic data pertaining to the distinction between active and inactive disease.

**Histology of orbital tissues during active and inactive disease stages.**

Naffziger operated on a number of patients with very severe, "malignant" ophthalmopathy and found pale, edematous tissue in patients with early disease, whereas those with longer standing ophthalmopathy had much fibrous tissue.\textsuperscript{69} In the early stages he found just swelling of the muscle fibers, during the "intermediate" stages a mononuclear cell infiltrate was observed, while in the late stages dense collagen scar tissue was prominent.\textsuperscript{69} Dunnington and Berke summarized their biopsy examinations also as the occurrence of a lymphocytic infiltrate, marked edema of muscle fibers, and fibrosis.\textsuperscript{70} Russell Brain contrasted histologic examinations in patients with short duration of disease (lymphocytic and plasma cell infiltrates, edema) to the findings at autopsy of a patient who had suffered from Graves' ophthalmopathy for 6\textfrac{1}{2} years.\textsuperscript{71} In this patient he saw massive fibrosis with the globe appearing to be anchored to the orbit by dense fibrous tissue strands. The eye muscles could only be identified as thin fibrotic bands. Daicker reported a chronic lymphocytic infiltration, edema, and fibroblast
activation in the biopsies from early, active patients, whereas in patients with longstanding ophthalmopathy only fibrosis and fat accumulation was seen. He also observed that the eye muscles could actually be more enlarged in inactive, than in active patients, and suggested that corticosteroids or radiotherapy are only effective during the active phase of fibroblast activation. Other studies have also noted the coexistence of a lymphocytic infiltrate with fibroblasts and so-called "young connective tissue". Tallstedt and Norberg found fibrosis, collagen deposition and activated fibroblasts in 4 patients with apparently inactive disease, whereas these abnormalities were also seen in a patient with rapidly progressive disease in whom, however, a more marked lymphocytic infiltrate was found.

Thus, the few histologic studies published do support the concept of Rundle’s curve. During the active stage there usually is edema, a lymphocytic infiltrate and activation of fibroblasts. In the end-stages, there is only fibrosis. It follows that to discriminate on histology between active and inactive disease, the presence of fibrosis is not helpful, since it is present throughout the disease process. One has to rely on the detection of edema and a lymphocytic infiltrate, which is however usually localized and its detection is thus liable to sampling errors.

Disease activity: therapeutical implications.
Daicker was the first to suggest that medical treatment (radiotherapy, corticosteroids) will only be effective during the active phases. For, both treatments are immunosuppressive and anti-inflammatory and will thus act on the lymphocytic infiltrate and edema of the active phase, and it is highly unlikely that they will improve the fibrous scar tissue left in the end-stages. However, end-stage ophthalmopathy can still cause considerable proptosis and diplopia, and this kind of patients will therefore seek help in specialized clinics. These inactive patients will not benefit from immunosuppression and should undergo rehabilitative surgery. Whereas a patient with the same degree of proptosis and diplopia in the active phase can be treated with steroids or irradiation, but surgery at that time
might actually be ineffective as any improvement of eye changes can be lost in the late postoperative stages due to ongoing expression of the disease.

Thus, the implications of the concept of disease activity are that the stage of the disease rather than the severity should dictate whether to use medical therapy or surgery. Obviously, this can be easy. A patient with rapidly progressive ophthalmopathy of six months duration will be treated medically. In contrast, a patient with diplopia, finally referred after 10 years, will be scheduled immediately for squint surgery. However, in many patients with Graves' ophthalmopathy this is much less obvious, and then an assessment of the activity might be helpful to decide on their treatment.

The concept of disease activity might also explain the fact that whichever medical treatment is given, only about two-thirds of the patients will respond. For, in most studies patients are recruited on the basis of the severity of their eye disease. Implying, that both active and inactive patients with similar severity are subjected to a medical treatment that will only benefit the active patients. If we were able to restrict our medical treatment to active patients only, the response rate to these therapies might increase considerably.

In conclusion, assessing the stage of the disease in individual patients will help us to decide on the most appropriate and logical therapy. Which is why many different groups have tried to develop methods to assess disease activity. Each of these parameters should however be validated.

### 1.3 Assessment of disease activity of GO

**Validation of activity parameters.**

It is not easy to assess whether a given parameter of disease activity actually reflects a lymphocytic infiltrate, edema and fibroblast activation. The proper control for this would be to relate the parameter to a biopsy of extraocular...
Chapter 1

muscles, or maybe connective tissue. For obvious reasons this approach is not feasible, because such a biopsy might be damaging to the patient. In addition, the biopsy would be liable to sampling error as described above. Thus the value of an activity parameter can not be related to a gold standard. And consequently, we have to accept a surrogate standard.

The surrogate criterion to be met by an activity parameter is its value in predicting the outcome of treatment. Why? Since the underlying concept is that the lymphocytic infiltrate etc of the active fase is amenable to medical intervention, an activity marker that is reflecting this ongoing immune process would be able to predict a beneficial response to immunosuppressive therapies. On the other hand, if an activity marker reflects fibrotic scar tissue this parameter would predict a negative outcome of immunosuppression. In other words if a marker has a high positive predictive value (+ve PV) for response to medical treatment, it detects the active patients. An activity marker with high negative predictive value (-ve PV; predicting no response) would be indicative of inactive burnt-out disease.

When there is reasonable biologic evidence that a particular test might be a marker of disease activity, this test should then be validated against the surrogate standard: the clinical outcome of treatment. Using a two-by-two table (figure 1.2) sensitivity, specificity, +ve PV and -ve PV can be calculated, indicating the clinical usefulness of the proposed test. One should however remember that this is a surrogate standard and subject to various confounding factors. Thus, the performance of a test will also depend on the way the clinical outcome is measured, on the efficacy of the treatment, and on the natural history of the disease. Since no treatment is always effective, the +ve PV will never be 100%. Also, because spontaneous improvement can occur during the beginning of the inactive fase, the -ve PV will never be 100% as well. Lastly, genetic factors might influence treatment outcome; several HLA markers have been shown to indicate good or bad response to medical therapy.75,76

Now we will review the different parameters for disease activity, and whether they have been tested in the above explained fashion. We will start with purely
clinical parameters, then focus on imaging techniques, and finally on serum markers.

Clinical assessment of disease activity.

The most obvious clinical parameter is the duration of the eye disease. Ophthalmopathy of short duration is likely still active, whereas long standing eye changes are more likely compatible with inactive disease. However, as stated above the duration of active ophthalmopathy is extremely variable (anywhere between several months to five years), and it is indeed the experience of most investigators that the mean duration of the eye disease is similar in responding and non-responding patients. Still, Donaldson et al did find a correlation between the response to prednisone and the duration of the exacerbation of the eye signs. Nobody has actually reported a 2x2 table analyzing its clinical usefulness. By compiling the individual patient data of two published papers from Pisa (Italy), such a table can be made (Fig. 1.2).

A second approach would be to just observe the patient over time. When there is progression, the disease is active; when the eye signs are stable for at least 6 months, the process is inactive. This approach has several drawbacks. First, in patients seeking help effective therapy is postponed. Secondly, this concept heavily leans on the power of our measurements to detect significant changes in disease severity. This power is certainly limited, especially in measuring soft tissue changes.

Trying to determine the activity of the eye disease by clinical examination is not new. Van Dyke proposed the mnemonic RELIEF, and in 1981 Sergott et al included clinical parameters in their activity score. None of these indexes were tested in a prospective study.

In 1989 a Clinical Activity Score (CAS) was proposed, which is based on the classical signs of inflammation: dolor, rubor, tumor, functio laesa (the fifth sign calor, heat, was considered but found unfeasible to measure). The CAS has one drawback: to evaluate impaired function, observation during two months is
necessary. Although the occurrence of a change in one to three months is relatively low (proptosis, 19%; motility, 7%; visual acuity, 2%), these individual signs do contribute greatly to the predictive value of the CAS. In the first, but retrospective analysis of the CAS, its performance was good. It was then tested in formal prospective study with the following results: a CAS of ≥4 had a positive PV of 80%, but a negative PV of 64%. Thus, although it is an useful and simple test, management decisions usually require other tests as well.

<table>
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<th>Responders</th>
<th>Non-Responders</th>
</tr>
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<tbody>
<tr>
<td>positive</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>negative</td>
<td>c</td>
<td>d</td>
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\[
\text{Cut-off} \quad \frac{a}{a+b} = +ve PV \quad \frac{d}{c+d} = -ve PV
\]

<table>
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<tr>
<th>Activity Test</th>
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<th>Non-Responders</th>
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<th>specificity</th>
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<tr>
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<td>68%</td>
<td>57%</td>
</tr>
<tr>
<td>negative</td>
<td>13</td>
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**Figure 1.2** Validation of a test determining disease activity of Graves' ophthalmopathy by its prediction of the response to immunosuppressive treatment (upper panel). The value of the duration of ophthalmopathy in predicting the outcome of immunosuppression, as compiled from patient data from two Italian studies (lower panel).
Introduction

Imaging techniques

magnetic resonance imaging (MRI)

MRI uses a very strong magnetic field to line up the protons (hydrogen atoms) in the body. Protons can be viewed as small magnetic bars with a north and south pole, which spin (actually whobble) around an axis, and by applying a gradient magnetic field the axes of the spins are lined up.\(^8\) Radiofrequency pulses from a radiofrequency transmitter coil (an antenna) bring the protons in a higher energy state, which will shift their spinning axes creating a radiofrequency field that can be detected by a receiver coil. The signal thus detected depends on the proton density of the tissue, and the T1 and T2 relaxation times of the tissues. The T1 time represents the rate at which the excited protons realign with the magnetic field. The T2 relaxation time represents another characteristic of the tissues. It is not determined by the axis of the spinning proton, but by the phase of the spin. The radiowave will also bring all spinning protons into the same phase (all north poles are pointing in the same direction). When the pulse is switched off, the protons start to dephase at a rate which is called the T2 relaxation time.\(^8\)

Especially the T2 weighted images have been found useful to detect edema and inflammation in extraocular eye muscles on MRI. Just et al described that a long T2 time in the eye muscles before treatment were associated with a good response to orbital irradiation.\(^8\) This first report was followed by others who confirmed that the T2 time decreases after immunosuppressive therapy.\(^8,8.9\) This suggests a transition from an edematous, inflammatory phase into a fibrotic end-stage. Indeed, Laitt et al found a correlation with the Clinical Activity Score and T2 times, using a more sophisticated protocol which suppresses the fat signal (short tau inversion recovery, STIR).\(^9\) Until now only one study assessed the value of T2 time measurements to predict the outcome of immunosuppressive treatment (intravenous steroids).\(^9\) In this small study in 23 patients, a +ve PV of 69% and a -ve PV of 86% was found. Thus, MRI seems a promising method to detect disease activity, but the good -ve PV awaits confirmation in a larger study.
Octreotide scintigraphy

Octreotide is an eight amino acid analog of the 14-aminoacid neuropeptide somatostatin with a prolonged half-life of 2-3 hours compared to the half-life of two to three minutes of native somatostatin. Somatostatin serves as a neurotransmitter and as a hormone by interacting with G-protein coupled somatostatin receptors widely distributed throughout the body. Octreotide can be radiolabeled and \(^{111}\text{In-DTPA-D-Phe1}\) octreotide is used in nuclear medicine to visualize tissues with somatostatin receptors. Activated lymphocytes express somatostatin receptors on the plasma membrane, and an attractive hypothesis would be that orbital uptake in GO is due to binding of \(^{111}\text{In-DTPA-octreotide}\) on these activated cells. However, binding to receptors on other cell types, or local blood pooling due to venous stasis are possible alternative explanations. Hypercirculation as a result of hyperthyroidism is unlikely to explain the orbital uptake, as a rather low orbital uptake was found in Graves’ hyperthyroid patients without clinical signs of ophthalmopathy. Postema et al were the first to show pronounced orbital octreotide uptake in patients with Graves’ ophthalmopathy. They also found that the orbital uptake was related to the clinical activity score and to the severity of GO with higher orbital uptake in more severe GO. Other investigators did not always find a correlation between orbital uptake and severity however, the orbital uptake was always correlated with the activity of GO. In inactive patients the octreotide accumulation is weak and resembles the uptake in normal orbits. The amount of octreotide uptake correlates also positively with the above mentioned T2 relaxation time on MRI.

Octreotide scanning of the orbits is usually done at 4 hours after injection and after 24 hours. The administered dose in the earlier studies was 222 MBq, but others have used approximately half of that dose and could still obtain a good image. The studies differ also in the time interval after i.v. injection, the selection of orbital slices for determining the number of counts in the orbit, and the method of correction for background radioactivity.
Octreotide uptake measurements might predict the outcome of immunosuppression.\textsuperscript{102} In a small, preliminary study with 12 patients Krassas et al found a +ve PV of 100\%, and a -ve PV of 83\%.\textsuperscript{103} Whether octreotide scintigraphy deserves a place in the diagnostic work-up of ophthalmopathy patients remains to be seen. However, it is an expensive diagnostic tool with a relatively high radioactivity burden.

**Ultrasound**

Ultrasound is an inexpensive method to visualize the orbital contents and has been used to measure the thickness of the eye muscles.\textsuperscript{41} However, it can also depict the internal echogenicity of the eye muscles which is best done using two-dimensional A-mode echography. The internal reflectivity of the soundbeam is low in patients with active eye disease, presumably due to edema, whereas the reflectivity is high and irregular in inactive patients, due to fibrotic echogenic scar tissue.\textsuperscript{104} In a preliminary study in 16 patients treated with radiotherapy, the method seemed promising in predicting a response to radiotherapy: positive predictive value of 73\% (95\%CI:39-94\%) and a negative predictive value of 100\% (95\%CI:48-100\%).\textsuperscript{104} Confirmation of these results is needed before ultrasonography can be considered as a disease activity parameter in GO.

**Laboratory measurements**

**Cytokines**

A key difference between the active and inactive phase of Graves’ ophthalmopathy is the lymphocytic infiltrate present in the orbital tissues during the active stage only. We know that lymphocytes produce cytokines, which can stimulate GAG production by fibroblasts, upregulate adhesion molecules and other immunomodulatory molecules like HSP-72 and HLA-DR.\textsuperscript{105,106} Measurements of these cytokines, their soluble receptors or of the molecules they upregulate might potentially differentiate the active from the inactive stage. However, there are some drawbacks to this approach. First, many of these molecules are also involved
in Graves’ thyroid disease, which might hinder their utility to detect activity of the orbital disease. Secondly, the orbits are small and even if cytokines are released into the bloodstream they might be difficult to detect. Thirdly, cytokines are largely bound to soluble receptors, carrier proteins and even autoantibodies in serum; blocking of epitopes used in sandwich immunoassays might hinder the detection of cytokines in serum.

Sergott et al measured sheep-erythrocyte Rosette formation by peripheral blood lymphocytes from patients with Graves’ ophthalmopathy and related the result to the outcome of steroid treatment. They found that the responsive (active) patients had a significantly decreased percentage of rosette formation.\textsuperscript{107} The number of spontaneous rosette-forming cells increases upon corticosteroid treatment in the responding patients, reaching values comparable to inactive and normal controls. Similar results were obtained by other investigators,\textsuperscript{108} but to our knowledge, presently this test is not in use.

The first ’cytokine’ measured was the Migration Inhibition Factor (MIF).\textsuperscript{109,110} Van der Gaag et al found that MIF was present in 11/18 (61%) of clinically active ophthalmopathy patients, compared to only 2/14 (14%; \( p=0.008 \)) in inactive patients.\textsuperscript{111} However, the MIF is measured in a rather cumbersome bioassay. Nowadays highly sensitive sandwich ELISA’s are being used to detect a fast growing number of cytokines.

Activated lymphocytes express IL-2 receptors and release a truncated form of this protein called the soluble IL-2 Receptor (sIL-2R). sIL-2R levels were found to be elevated in patients with severe ophthalmopathy (in 21 out of 47 patients = 45%),\textsuperscript{112} and this correlates with disease activity.\textsuperscript{113} However, the clinical usefulness of sIL-2R levels is limited with a +ve PV of only 71%, and -ve PV of 54%.\textsuperscript{113} IL-6 is produced by lymphocytes, but also by fibroblasts and tumor cells and is elevated in serum from patients with Graves’ hyperthyroidism.\textsuperscript{114} Its soluble receptor (sIL-6R) is elevated as well in hyperthyroidism, but was also found to be related to the activity of the ophthalmopathy.\textsuperscript{114} However, because of the huge overlap in sIL-6R values between active and inactive ophthalmopathy patients it is
unlikely to be helpful to diagnose activity in individual patients. CD30 is a protein expressed on the membrane of activated T-helper 2 cells, and a soluble form (sCD30) released by proteolytic cleavage can be measured in serum. It was found to be elevated in patients with active Graves' thyroid disease, correlating with the titer of TSH-R antibodies. It is unknown whether ophthalmopathy might be another determinant of sCD30 levels.

IL-1 is also a potent stimulator of GAG production by fibroblasts, and this can be inhibited by IL-1 Receptor antagonist and by the soluble IL-1 receptor (sIL-1R). The soluble form of the IL-1 receptor antagonist (sIL-1RA) and the sIL-1R can be measured in serum, and it was hypothesized that higher levels of these IL-1 inhibitors would protect the patient with ophthalmopathy. Indeed, it was shown that patients who responded well to radiotherapy had higher baseline sIL-1R and sIL-1RA levels than those who did not respond. Also, in the responders a significant increase in these levels occurred after radiotherapy, which did not occur in the nonresponders. The predictive values of sIL-1R and sIL-1RA were not reported, but the authors also found that the levels of these IL-1 inhibitors were lower in smoking than in non-smoking patients.

Until now no single cytokine was found to reliably predict the outcome of immunosuppression. Cytokines act in a network, and this network might regulate itself through other cytokines. It might well be that we would have to measure a number of different cytokines, or a ratio of 'stimulating' to 'inhibitory' cytokines to get an impression of the activity of a disease. However, 'new' cytokines are discovered regularly and it might be worthwhile to just wait till the network is fully unravelled.

**Immunomodulatory molecules**

Cytokines and other factors (including immunoglobulins) have been shown to upregulate various adhesion molecules on orbital fibroblasts. Adhesion molecules play an important role in directing the traffic of circulating lymphocytes towards their target: homing. They are functioning as receptors between
immunocompetent cells, connective tissue and extracellular matrix components.\textsuperscript{120} Intercellular adhesion molecule-1 (ICAM-1) is such an adhesion molecule abundantly present in orbital tissue of active ophthalmopathy patients.\textsuperscript{121,122} Soluble forms of ICAM-1 (sICAM-1) are shedded into the circulation and might therefore reflect the expression of ICAM-1 in the orbit. Cytokines (IFN-\(\gamma\), TNF-\(\alpha\), IL-1\(\alpha\)) strongly enhance surface expression of ICAM-1 in both retro-ocular fibroblasts of normals and GO.\textsuperscript{123} Heufelder and Bahn found elevated levels of sICAM-1 in sera of patients with active eye disease (compared to patients with no, or inactive ophthalmopathy) and found that sICAM-1 levels decrease upon prednisone treatment.\textsuperscript{124} Their findings were recently confirmed by De Bellis et al.,\textsuperscript{125} who showed that patients with active ophthalmopathy had significantly increased levels of sICAM-1, although Graves' hyperthyroid disease patients without eye involvement also had higher levels than controls, albeit still lower than the ophthalmopathy patients. This might be due to the fact that ICAM-1 is expressed in thyroid tissue as well as in orbital tissues in Graves' disease. Another adhesion molecule, endothelial-leucocyte adhesion molecule-1 (ELAM-1) is found in orbital tissues but not in the thyroid and the same group reported clearly elevated sELAM-1 levels in ophthalmopathy patients, but not in patients with Graves' thyroid disease without eye involvement.\textsuperscript{125} According to their data, there was even no overlap between sELAM-1 levels in ophthalmopathy patients and hyperthyroidism patients, and the levels had a correlation with the CAS (\(r=0.55\); \(P<0.002\)).\textsuperscript{125} Another molecule upregulated by cytokines in orbital tissue is heat shock protein-72 (HSP-72), which is able to induce an immune response. Indeed, anti-HSP72 antibodies have been found in sera from patients with Graves' disease, but the levels did not correlate with disease activity.\textsuperscript{126} Serum HSP72 levels have not been measured in Graves' disease patients.

Autoantibodies

Since we do not know the antigen responsible for the autoimmune attack in the orbit, disease specific autoantibodies cannot be measured yet. There was some
Introduction

hope that antibodies against a 64 kD eye muscle protein might reflect the activity of the eye disease, but we now know that it is not an eye muscle specific protein and that 20% of normal controls also have anti-64 kD autoantibodies, and the value of determining these antibodies has been questioned seriously.\textsuperscript{127} Recent evidence for the presence of the TSH-R in the orbit\textsuperscript{128-132}, has given new life to the old hypothesis that TSH-R autoantibodies might in fact cross-react with the thyroid and orbit and be the link for the two manifestations of Graves’ disease. Patients with ophthalmopathy indeed have higher levels of TSH-R autoantibodies,\textsuperscript{133} but in older studies they do not seem to correlate with clinical characteristics of the eye disease.\textsuperscript{134}

\textit{Glycosaminoglycans (GAG)}

GAG’s play a key role in the manifestations of Graves’ ophthalmopathy. They are hydrophilic proteoglycans which attract water and are the main reason for the increase in the volume of orbital tissues.\textsuperscript{21} They are produced by activated fibroblasts,\textsuperscript{135} and therefore GAGs might serve as a marker of active eye disease. GAGs are present in plasma and urine also in healthy controls, and are thus not specific for Graves’ ophthalmopathy. In a recent study, Pappa et al found no correlation between serum hyaluronan levels and hyaluronan tissue levels.\textsuperscript{21} Nevertheless, Kahaly et al found that ophthalmopathy patients have higher urinary GAG levels compared to controls and to patients with Graves’ hyperthyroidism without eye disease.\textsuperscript{136} Though there was a considerable overlap, a cut-off value can be calculated that discriminate active from inactive patients (assessed on clinical criteria): +ve PV 68%, -ve PV 96%. Others could, however, not confirm their findings and found similar urinary GAG excretion in ophthalmopathy patients and controls.\textsuperscript{137} In later studies the group of Kahaly used a HPLC method to determine GAG levels, which improved the detection considerably.\textsuperscript{138} Using this method, they again found higher levels of GAG excretion in active patients compared to inactive patients.\textsuperscript{139} They also reported significantly elevated plasma GAG levels in patients with active, untreated ophthalmopathy, whereas plasma
Chapter 1

GAG levels were normal in patients with treated, inactive eye disease. However, its real predictive value for the therapeutic outcome after immunosuppression has not yet been published.

1.4 Outline of the present thesis

This thesis deals with the assessment of disease activity and its usefulness in predicting outcome of immunosuppression in Graves' ophthalmopathy. We determined various parameters for disease activity in a cohort of 66 patients with Graves' ophthalmopathy, who qualified for retrobulbar radiotherapy because of the severity of their eye disease. Using predefined criteria we included patients with so-called moderately severe ophthalmopathy, excluding patients with sight loss because they needed a stronger immunosuppressive regimen or immediate decompressive surgery, as well as patients with minor ophthalmopathy, in whom the rationale for immunosuppressive treatment not yet has been established. The 66 patients were thus selected on the basis of the severity of their eye disease, and not because of the activity of their eye disease.

In all patients a variety of putative activity parameters were measured before the start of the irradiation. Six months after this treatment the therapeutic outcome was carefully determined on the basis of predefined criteria. Responders to radiotherapy were assumed to have been active before start of treatment, non-responders were thought to have been inactive at the time of irradiation. The various activity parameters were then related to the therapeutic outcome and it was analyzed whether the pretreatment activity parameters could predict the outcome of radiotherapy. Therefore, we used the surrogate standard as discussed above.

Chapter 2. Urinary glycosaminoglycan excretion (GAGs) was shown to be promising in determining disease activity. It was measured in two consecutive 24 hour urine collections prior to the start of radiotherapy by conventional methods as
well as by high pressure liquid chromatography (HPLC) (Laboratory of Prof Dr G. Kahaly, Mainz, Germany). The pretreatment values were analysed using receiver operating characteristic (ROC) curves, from which a cut-off value was determined to calculate sensitivity, specificity, positive and negative predictive values.

Chapter 3. A panel of several cytokines and soluble cytokine receptors was measured in pretreatment serum of our ophthalmopathy patients. In order to establish whether these levels were different from healthy subjects, these cytokines were also measured in 60 healthy controls matched for age, sex and smoking behaviour. The cytokine levels were then compared to the outcome of irradiation, also in a multivariate analysis.

Chapter 4. TSH-receptor stimulating antibodies are the cause of Graves’ hyperthyroidism by stimulating the thyroidal TSH-receptor. There is increasing evidence that the TSH-receptor is also present in orbital tissues (most notably the retro-orbital fibroblast). If so, TSH-receptor autoantibodies might cross react with this orbital TSH-receptor, which might play a causative (or permitting) role in the pathogenesis of Graves’ ophthalmopathy. To find support for this hypothesis we measured TSH-Binding-Inhibiting Immunoglobulins (TBII using the TRAK-assay), and TSH-Receptor Stimulating Immunoglobulins (TSI measuring the cAMP response in a bioassay) in our patients with ophthalmopathy. The TBII/TSI levels were related to the severity of the eye disease, but also to the activity (Clinical Activity Score, CAS). In addition, we analyzed whether TBII or TSI pretreatment levels could predict therapeutic outcome of radiotherapy.

Chapter 5. In a preliminary study, the determination of the internal eye muscle reflectivity by A-mode ultrasonography was found to be promising in predicting the therapeutic outcome in 16 ophthalmopathy patients. We therefore performed this inexpensive technique in our cohort, and determined the ability of ultrasound
to predict the outcome of irradiation, and analyzed the findings by calculating the area under the ROC-curve.

**Chapter 6.**

Magnetic resonance imaging (MRI) uses a magnetic field to line up the protons (hydrogen atoms) in the body. A method to estimate a real T2 time within the extra-ocular eye muscles, corrected for a relatively high content of fat (white matter of the central nervous system, or retrobulbar fat) is described. The quantitative MRI might be useful in assessing (indirectly) oedema within the eye muscles. The larger the amount of oedema, the longer the T2 time, indicating active inflammation.

**Chapter 7.** $^{111}$In-DTPA-octreotide is a radioactive labeled long acting somatostatin analogue, and has been used to visualize lymphocytic infiltration of retrobulbar tissues. Activated lymphocytes express somatostatin receptors on the plasmamembrane and one hypothesis is that the orbital uptake in GO is due to binding of $^{111}$In-DTPA-octreotide to activated T-lymphocytes. In this review on octreotide scintigraphy we discuss the determinants of thyroidal and orbital uptake of radiolabeled octreotide and discuss the precision and accuracy of orbital radiolabeled octreotide accumulation as determined in various studies.

**Chapter 8.** In view of the promising results reviewed in chapter 7, we used this expensive technique in our patients. In order to be able to analyze the $^{111}$In-DTPA-octreotide scintigraphy data, we first performed a preliminary study in 22 patients from our cohort. The aim of this study was to determine the best method to correct for background uptake (the occipital skull, or the temporal region), and to establish which of the two scans (4 hours, or 24 hours post injection) was the most reliable in terms of intra-observer variation and in terms of predicting therapeutic outcome.
Introduction

Chapter 9. In the preceding chapters, various parameters were tested alone in a univariate analysis. In this chapter we developed a logistic, multivariate regression model using all of the above described activity tests in combination with additional markers of disease activity (duration of the eye disease, CAS, MRI). From this model a Response Score can be calculated, which can be used in individual patients to predict their chance of responding to immunosuppressive therapy.

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Introduction


