Graves' ophthalmopathy: in search of better markers and better treatment

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Chapter 1

General introduction
1.1 Introduction to Graves’ ophthalmopathy

Graves’ ophthalmopathy (GO), also known as Graves’ orbitopathy or thyroid associated ophthalmopathy, has crippled many patients, and intrigued many physicians, endocrinologists and ophthalmologists alike. The combination of goiter, palpitations and exophthalmos was first described in the 19th century by Robert Graves and baron Von Basedow, and is nowadays usually referred to as Graves’ disease.1,2 Graves’ ophthalmopathy is of presumed autoimmune aetiology, and closely connected to Graves’ hyperthyroidism which is caused by autoantibodies against the TSH-Receptor (TSH-R).3,4 Despite the fact that these days much is known about the immunopathogenesis of Graves’ ophthalmopathy and the following sequence of clinical events, our present knowledge is still insufficient to prevent this disease, notwithstanding the progress that has been made.

1.2 Clinical presentation

There is an enormous variability in the signs and symptoms of patients presenting with Graves’ ophthalmopathy (see Table 1 the NO SPECS classification of eye changes). Some patients complain about photophobia or a gritty sensation in the eyes only, whereas others have complaints of severe facial disfigurement due to swelling of the eye lids, eyelid retraction, visible squint and/or protruding eye balls (Figure 1A–G). Other complaints consist of double vision or disturbed visual acuity and retrobulbar pain at rest or with movement. Patients with GO usually suffer from Graves’ hyperthyroidism as well, although about 20% of patients are euthyroid and 5% are diagnosed with primary hypothyroidism.3,4 On the other hand, approximately 25% of patients with Graves’ hyperthyroidism show signs and symptoms of Graves’ ophthalmopathy.3,4

1.2.1 Classification of eye changes In this thesis the clinical features of Graves’ ophthalmopathy are classified according to the NOSPECS classification (Table 1).5-10

CLASS 1. ONLY SIGNS, NO SYMPTOMS

Upper eyelid retraction is often observed in patients with Graves’ hyperthyroidism and less frequently in patients with thyrotoxicosis due to toxic multinodular goiter.11,12 This retraction causes lid lag on downward gaze (Von Graefe’s sign) and a staring gaze. This lid retraction can be due to swelling of the levator muscle or can be the result of sympathetic tone increase in thyrotoxicosis. It is also possible that adhesions around the levator muscle are a cause of eyelid retraction, as patients who are rendered euthyroid often still have this feature.13 Lid aperture can be measured in mm using a simple ruler.14

CLASS 2. SOFT TISSUE INVOLVEMENT

This consists of swelling of the upper and lower eyelids, swelling of the caruncle, chemosis (edema of the conjunctiva) and conjunctival injection and redness. The increase in retrobulbar tissues (both extraocular muscle and fat) within the bony surroundings
the human orbit leads to increased space occupancy, and in combination with the confined space of the bony orbit to increased intraorbital pressure (Figure 2).\textsuperscript{15,16} A tight orbital septum may increase intraorbital pressure even further.\textsuperscript{17} This results in impaired venous drainage with periorbital edema and chemosis. An alternative explanation might be herniation of retrobulbar tissue through the naturally occurring holes in the orbital septum.\textsuperscript{17} The extent of soft tissue involvement can be scored using colour slides or using a colour atlas.\textsuperscript{18,19}

\section*{Class 3. Proptosis}

The increased retrobulbar pressure pushes the globe forward, causing exophthalmos. Rundle and Pochin have demonstrated in post mortem studies that the normal orbital cavity has a volume of about 26 ml, and normal eye muscle volume is about 3.5 ml.\textsuperscript{20} If the eye muscle volume increases by 4 ml this leads to a proptosis of 6 mm. In other words, relatively small changes in tissue volume can cause major changes in proptosis. One of the limitations of the NO SPECS system is that proptosis is underestimated in this classification. Proptosis is usually measured in mm, using a Hertel exophthalmometer, or can be measured on CT-scan (Figure 1).\textsuperscript{14} Hertel values of 20–22 mm are not scored although these can reflect severe increases in proptosis in individual patients. In the Netherlands, Hertel values in healthy females range from 10–16 mm (mean 12.6 mm) and in healthy males from 9–18 mm (mean 13.9).\textsuperscript{21}

\section*{Class 4. Extraocular muscle involvement}

Swelling of the extraocular muscles leads to diminished eye muscle motility. The superior, medial and inferior muscles are usually affected and the lateral muscles are often spared.\textsuperscript{3} The motility impairment of the extraocular muscles is caused by restricted relaxation of the affected antagonist, while looking in the opposite direction. This can be appreciated with the "forced duction test".

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Figure 1 Panel A: patient with visible squint; Panel B: patient with unilateral proptosis due to extraocular muscle enlargement; Panel C: corresponding orbital CT-scan; Panel D: patient with optic neuropathy; Panel E: severe edema and chemosis; Panel F: patient with eyelid retraction and marked proptosis due to increased intraorbital fat; Panel G: corresponding orbital CT-scan.

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Figure 2 Upper panel: sagittal selection through the right orbital cavity. Lower panel: the muscles of the right orbit, lateral aspect. (Reproduced with permission from the Publisher.)\textsuperscript{14,27}
Table 1 Modified NO SPECS classification of eye changes in Graves' disease.5-10

<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>Soft tissue involvement</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>4</td>
<td></td>
<td>Extracocular muscle involvement</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Corneal involvement</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>Sight loss due to optic involvement</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade</th>
<th>Suggestions for grading</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grading according to diplopia</td>
</tr>
<tr>
<td>0</td>
<td>absent</td>
</tr>
<tr>
<td>a</td>
<td>intermittent (when fatigued)</td>
</tr>
<tr>
<td>b</td>
<td>inconstant (present but not in primary gaze)</td>
</tr>
<tr>
<td>c</td>
<td>constant (present in primary gaze)</td>
</tr>
<tr>
<td></td>
<td>absent</td>
</tr>
<tr>
<td>a</td>
<td>limitation of motion at extremes of a gaze</td>
</tr>
<tr>
<td>b</td>
<td>evident restriction of motion</td>
</tr>
<tr>
<td>c</td>
<td>fixation of a globe or globes</td>
</tr>
<tr>
<td></td>
<td>absent</td>
</tr>
<tr>
<td>a</td>
<td>stippling of cornea</td>
</tr>
<tr>
<td>b</td>
<td>ulceration</td>
</tr>
<tr>
<td>c</td>
<td>clouding, necrosis, perforation</td>
</tr>
<tr>
<td></td>
<td>absent, vision &gt;0.8</td>
</tr>
<tr>
<td>a</td>
<td>Disc pallor, visual yield defects, vision 0.5–0.63</td>
</tr>
<tr>
<td>b</td>
<td>Same, but vision 0.1–0.4</td>
</tr>
<tr>
<td>c</td>
<td>Same, but vision &lt;0.1–blindness</td>
</tr>
</tbody>
</table>

By actually grabbing the globe and attempting to move it in the direction the patient cannot, mechanical resistance is encountered. Since the inferior muscle is most commonly involved, limitation of elevation is a common finding.22 With diminished eye muscle motility, a so-called torticollis oculi (head tilt) may arise. This may be illustrated when we picture a patient whose gaze points downwards and hence lays the head backwards in order to look up. When the increase in muscle volume is asymmetrical between the right
and left eye, thus influencing relative eye motility differences, complaints of double vision may arise, which is a quite invalidating condition. It should be noted that diplopia will rarely be found when visual acuity is almost absent in one eye.

Although the muscle volume increase in GO is the most striking and can easily double, there may also be an increase in orbital fat. Forbes et al. measured orbital fat and muscle volumes on CT-scans of the orbit of 22 healthy subjects and 72 Graves' patients. They found increased volumes in GO patients when compared with controls.

In 49% of patients this increase consisted of an increase in both muscles and fat volume whereas in 39% of patients it was caused by an increase of only muscles and in 11% by an increase of only fat. Whether fat increase represents a distinct entity is currently debated. Disturbances in extraocular muscle motility can be measured quantitatively using different techniques. Mourits et al. have developed a reliable and reproducible technique using a modified hand perimeter to measure eye muscle motility in degrees. Diplopia can be easily graded in the outpatient clinic using the Gormann score (Table 1).

**Class 5. Corneal involvement**

Corneal overexposure is the result of exophthalmos, eyelid retraction and reduced blinking. Impaired motility of the inferior eye muscle and decreased quality and quantity of tear production may also contribute to corneal irritation. This situation may lead to painful keratopathy and, when not recognized, to corneal ulceration, especially when the eyes do not close at night due to proptosis (lagophthalmos and exophthalmos). Sight loss caused by corneal involvement should be distinguished from sight loss due to optic nerve involvement. Early symptoms of corneal involvement are a gritty sensation, blurred vision and intolerance to contact lenses. These symptoms can be readily relieved by using artificial tears, eye ointments and sunglasses.

**Class 6. Optic nerve involvement**

Optic neuropathy is due to direct compression of the optic nerve caused by swelling of the extraocular muscles near the apex of the orbit, seen as 'apical crowding' on CT-scans. It is the result of very high retrobulbar pressure that is not sufficiently relieved by developing exophthalmos if not adequately treated, it may lead to blindness. Otto et al. have shown that the intraorbital pressure just before decompressive surgery in patients with optic neuropathy ranged from 12–40 mm Hg (mean 29 mm Hg) and was reduced by 8–12 mmHg after surgery. In contrast the intraorbital pressure in patients decompressed for rehabilitative reasons (marked exophthalmos without optic neuropathy) was 9–11 mm Hg, and did not change after surgery. Patients who suffer from optic neuropathy have relatively low Hertel readings. Koornneef suggested that severe eye disease with optic nerve involvement is due to well developed and firm connective tissue in these patients. Therefore these patients do not develop proptosis (which can be considered to be nature's own decompression) but rather develop sight loss due to increased retrobulbar pressure (Figure 3).

Measuring visual acuity is somewhat subjective. In Graves' ophthalmopathy one should distinguish sight loss due to optic neuropathy from sight loss from other conditions such as...
strabismus, cataract, keratopathy, retinopathy etc. Sight loss caused by optic neuropathy is often accompanied by visual field defects, impaired colour vision and a delayed latency on visual evoked potential. Papillary edema and disk pallor can be seen. So called ‘apical crowding’ on orbital CT-scans is a risk factor for optic neuropathy. Pinhole visual acuity can be measured using the Snellen Chart (decimal system).

To give an idea about frequencies of the different NO SPECS classes: in a European cohort of 152 newly referred GO patients it was found that 75% of patients had eye lid swelling, 38% had proptosis ≥ 23 mm, 49% suffered from diplopia, 16% corneal involvement and 21% optic nerve involvement.16

![Figure 4](image4.png)

Figure 4 “Rundle’s curve”, describing the natural course of the eye disease over a variable period of several months to a few years, adapted by M.F. Prummel.143

1.2.2 Quality of life GO is often an invalidating disease to the patient. Not only visual disfunction but also facial disfigurement has a major impact on daily functioning and well-being. Bartley et al.31 reported that after treatment 61% of patients believed that the appearance of their eyes had not returned to baseline, 51% believed their eyes still were abnormal in appearance and 37% was dissatisfied with the appearance of their eyes. Gerdinger et al. found a decreased general quality of life in 70 euthyroid GO patients compared with a reference population of 2595 healthy Americans.31 GO patients scored 8–36% lower on all scales of the MOS-24, except for ‘bodily pain’. GO patients also scored worse than patients with chronic conditions such as diabetes mellitus or emphysema, but comparable with Dutch patients suffering from Crohn’s disease. A disease-specific quality of life questionnaire for patients with GO, the GO-QOL, has been developed by our group.32 This questionnaire contains 16 questions, eight of which deal with the consequences of impaired visual functions and eight questions deal with the consequences of changed appearance. This GO-QOL has been validated and reliable measured changes over time in visual functioning and appearance of patients with GO.33,34 The GO-QOL can be a useful instrument for the outcome of the disease in terms of quality of life in clinical trials.

1.3 Natural course: disease severity and activity

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

Rundle followed 12 GO patients and found that the eye disease starts with a dynamic phase, which is characterized by aggravations and remissions, followed by a static phase (Figure 4).35 The disease could still be severe when the static stage was reached. Rundle’s curve is generally accepted, even though the time axis varies widely between patients and may read anywhere between some months and several years. It was noted that while the disease tends towards spontaneous regression, a return to the normal, premorbid state was seldom reached. These observations were later confirmed.36,37 Perros et al. found that spontaneous improvement occurred in 64% of 59 GO patients
Fig. 5 Concept of disease activity. Panel A shows GO disease severity (continuous line) over time. GO starts, reaches a maximum (100%) and then subsides, although premorbid stages are seldom reached. The dotted line represents disease activity. Panel B shows the result of immunosuppressive interference at 50% disease severity (open dot), but initiated after the disease has reached the fibrotic endstage, so no effect can be expected. Panel C shows the result of immunosuppressive interference, again at 50% severity level (open dot, followed by continuous line), but when GO activity is 100%. In this stage, therapy is useful to prevent further deterioration.

during a median follow-up of 12 months, while the disease remained stable in 22% and progressed in 14% of cases. These results were biased because it concerned a group of selected GO patients in whom improvement rate was probably overestimated. Regardless of the fact that the majority of patients seem to improve, most patients feel that their eyes never return to normal. Naffziger operated on a number of patients with very severe ophthalmopathy and found edematous tissue in patients with early disease, whereas those with longstanding ophthalmopathy had much fibrous tissue. 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. Brain found edema and lymphocytic cell infiltrates in patients with a short disease duration in contrast to massive fibrosis in a patient who suffered from GO for more than six years. So the concept of Rundle’s curve is supported by histological studies. During the active phase there usually is edema, a lymphocytic infiltrate and activation of fibroblasts. In the end-stages there only is fibrosis. This concept implies that interference with immunosuppressive therapy is only useful in the “active” inflammatory phase (Figure 5A–C). In fact, Daicker was the first to suggest that medical treatment such as glucocorticoids or radiotherapy will only be effective during the active phase. Because both treatments have immunosuppressive and anti-inflammatory effects and hence can interact with the lymphocytic infiltrate and edema. It is very unlikely that these treatments will change the fibrous scar tissue that is left in the end stages of GO. However, these patients can still have considerable proptosis and diplopia, and because they are less likely to benefit from immunosuppressive drugs should undergo rehabilitative surgery. This confronts clinicians with the problem to discriminate patients with active GO, in other words patients who will benefit from immunosuppressive therapy, from patients with endstage disease. In some cases this is not very difficult, but in others this will pose a dilemma. The concept of disease activity might explain that in most prospective trials
Table 2: The 10 items of the Clinical Activity Score (CAS). For each item, one point is given. The sum of these points is the CAS.45

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>1 Painful, oppressive feeling behind the globe, during the last four weeks</td>
</tr>
<tr>
<td></td>
<td>2 Pain on attempted up-, side- or downward gaze</td>
</tr>
<tr>
<td>Redness</td>
<td>3 Redness of the eyelids</td>
</tr>
<tr>
<td></td>
<td>4 Diffuse redness of the conjunctiva, covering at least one quadrant</td>
</tr>
<tr>
<td>Swelling</td>
<td>5 Swelling of the eyelids</td>
</tr>
<tr>
<td></td>
<td>6 Chemosis</td>
</tr>
<tr>
<td></td>
<td>7 Swollen caruncle</td>
</tr>
<tr>
<td>Impaired function</td>
<td>8 Increase in proptosis of 2 mm or more during a period of 1–3 months</td>
</tr>
<tr>
<td></td>
<td>9 Decrease of eye movements in any direction of 5 degrees or more during a period of 1–3 months</td>
</tr>
<tr>
<td></td>
<td>10 Decrease of visual acuity of one line or more on the Snellen chart (using a pinhole) during a period of 1–3 months</td>
</tr>
</tbody>
</table>

with medical treatment there is a response in circa 60–70% of patients.43,44 Because, in most studies patients are recruited on the basis of severity of eye disease and not activity and only patients who had active GO could benefit from medical treatment. It would be preferable to select active patients for immunosuppressive treatment to increase the response rate and not submit patients who will not benefit from such a treatment to potentially serious side effects.

The question arises whether and how activity can be measured. In contrast to other autoimmune diseases such as Crohn’s disease or rheumatoid arthritis, it is hardly possible to obtain a histological biopsy of the affected tissue in order to determine the level of inflammation. For this reason, the Clinical Activity Score (CAS) has been developed and tested on GO patients. This classification is based on the classical signs of inflammation: pain, redness, swelling and impaired function.45 The CAS consists of ten items, for each criterion met one point is scored (Table 2). In a retrospective study, Mourits et al.46 have found (studying 26 patients with severe GO) that a CAS of 3 or more was associated with "active" disease, and that these GO patients responded well to immunosuppressive therapy as opposed to those who had a lower CAS and were deemed "not active". This has been confirmed in a prospective study in 43 patients.46 Using a cut-off for the CAS of ≥ 4 the accuracy to predict therapeutical outcome was: specificity 86%, sensitivity 55%, positive predictive value 80% and negative predictive value 64%. The CAS remains a subjective marker of disease activity, and therefore a better and more objective disease activity marker would be helpful in order to select which patients could benefit from immunosuppressive therapy. Chapter 2, 3 and 4 discuss the search for a marker of disease activity. Disease duration can be used as an activity marker as well, although it should be used with caution. Disease duration, counted from the first signs and symptoms in combination with the CAS can be helpful. Other activity parameters such as ultrasound examination of extraocular muscles or octreotide scintigraphy have not been used in this thesis and will not be discussed further.
1.4 Etiology

Graves' disease is an autoimmune disease with an unknown and probably multifactorial etiology. There is certainly a genetic predisposition, since Graves' disease runs in families. Many candidate genes have been investigated and some have been shown in association studies to confer susceptibility for Graves' disease. Detailed analysis of Danish twin cohort studies has shown that the concordance rate for Graves' disease in monozygotic twins is 0.35 compared to 0.03 in dizygotic twins, and that by model-fitting analysis about 70% of the likelihood of developing Graves' disease is attributable to genetic factors and 30% to environmental factors. Recently, genetic polymorphisms in the CTLA4 gene which are thought to be associated with the risk to develop autoimmune disease have been found, Graves' disease being such an autoimmune disorder. Certain polymorphisms in the CTLA4 gene have been found to be associated specifically with Graves' ophthalmopathy whereas others do not differ between Graves' patients with or without ophthalmopathy. In the years to come, detailed molecular studies may shed light on the genetic background of Graves' ophthalmopathy. Yet, the genetic predisposition is not at all clear cut, and it appears that environmental factors are important triggers for the development of GO. Stressful life events are well known triggers for the development of Graves' disease, probably in genetically susceptible subjects. A number of other environmental factors have been investigated or are currently under investigation, but the best known risk factor for the development of Graves' ophthalmopathy is smoking. The proportion of smokers among GO patients is much higher than in patients with Graves' disease without GO or in the general population. Prummel et al. found an Odds Ratio of 7.7 (95% C.I. 4.3–13.7) for smokers to develop Graves' ophthalmopathy. Smoking was found to be associated with Graves' hyperthyroidism also, although to a lesser extent (Odds Ratio 1.9; 95% C.I. 1.1–3.2). Others have confirmed these findings with a mean Odds Ratio of around three. There is a positive association between the amount of cigarettes consumed and the increased risk of GO. There is also a positive correlation between the severity of GO and the amount of tobacco consumption. Moreover, patients who continue smoking benefit less from treatment of their eye disease than patients who quit their habit. How smoking contributes towards the elevated risk at developing GO is not known. There might be a role for hypoxemia as was shown by Metcalfe et al., who demonstrated in vitro that orbital fibroblasts from healthy individuals produce more glycosaminoglycans (GAGs) and synthesize more DNA when under hypoxic conditions. Smoking enhances the generation of superoxide radicals and reduces the formation of antioxidants. It could also be that smoking induces a general increase in tonus of the immune system. A combination of nicotin and tar with IFN-γ led to upregulation of HLA-DR in orbital fibroblasts of GO patients. The titer of anti HSP72 antibodies was increased in smoking subjects and Graves' thyroid disease patients. In our studies we have considered the influence of smoking on serum concentrations of cytokines and adhesion molecules.

1.5 Immunopathogenesis

1.5.1 Introduction Graves' thyroid disease is an autoimmune disorder caused by stimulating autoantibodies against the TSH Receptor. The hypersecretion of thyroid hormones is induced by binding of these autoantibodies to the TSH-R. The closely linked Graves' ophthalmopathy is presumably an autoimmune disorder as well. The autoantigen
responsible for this autoimmune attack is still not defined, but there is accumulating evidence that the TSH-R plays an incompletely understood role in the initiation or perpetuation of the orbital autoimmunity.

1.5.2 Autoimmunity Autoimmune diseases result from the breakdown of self-tolerance that protects healthy individuals from potentially harmful effects of autoreactive B and T cells. All humans possess such autoreactive cells. In the absence of antigen driven differentiation of effector cells there is no attack from the autoreactive cells to an organ or target cell. T cells can be divided in Th1, Th2 and T regulatory cells, based upon their function and the cytokines they produce. Th1 cells produce cytokines such as IFN-γ and IL-2, and Th2 cells produce IL-4 and IL-13. There are different subsets of T regulatory cells (all of which are CD4+). IL-10 is an important cytokine produced by T regulatory cells. These subsets of T cells with their unique cytokine profiles function within an intricate network. The initial step in an autoimmune response against a T cell-dependent antigen is phagocytosis of the antigen by antigen presenting cells (APC’s), i.e. dendritic cells. There is accumulating evidence that T and B cells are under the control of APC’s which thereby determine immunity and tolerance. Once they pick up the antigen, dendritic cells migrate and process the antigen in the T cell zone of the regional lymph node. Antigen specific T cells are trapped by dendritic cells with relevant MHC class I and II peptides exposed on their surface. Subsequently, circulating B cells are trapped in the lymph node and stimulated by T cells to produce antibody. T cells then proliferate and differentiate into T helper and T effector cells, each with unique functions and cytokine profiles.

1.5.3 Autoimmune process in GO The autoimmune process in the orbit is initiated by invasion of T cells and plasma cells. Adhesion, recruitment and extravasation of mononuclear cells take place when lymphocytes have been activated by antigen. The local expression of certain adhesion molecules functions as a key regulatory mechanism for a variety of effector cell functions at the site of an inflammatory or immune process, in this case the orbital tissues. These functions include the activation and recruitment of mononuclear cells to specific tissue sites, their migration and targeting in the extravascular space, as well as the presentation and recognition of antigens. This process is known as homing of T cells. Different celltypes, among which fibroblasts can express adhesion molecules such as ICAM-1, on the cell membrane in response to cytokine stimulation and via this route recruit new inflammatory cells. T cells express LFA on their cell surface and at an already inflamed tissue site, are capable of perpetuating the autoimmune process. Under the influence of cytokines and other immunomodulatory molecules, fibroblasts proliferate and differentiate and GAG production is stimulated. These GAG’s are hydrophilic in nature and cause edema by attraction of water. This leads to swelling of orbital tissue, both muscle and fat, and causes the clinical manifestations of proptosis, diplopia, periorbital swelling and redness.

1.5.4 Pathology Histological examination of orbital tissue in the early stages of Graves’ ophthalmopathy shows a lymphocytic infiltrate consisting primarily of T-helper and T-suppressor cells, together with some B-cells, macrophages and a few plasma cells and mast cells. Once the active inflammation has subsided fibrotic tissue is left. Brain et al. contrasted histologic examinations from patients with short disease duration to the findings at autopsy of a patient who had suffered from longstanding GO (6.5 years). They found lymphocytic and plasma cell infiltrates with edema in the first and massive fibrosis in the latter. Others have confirmed
these findings. Daicker also found that the eye muscles could actually be more enlarged in patients with an inactive and longstanding disease than in patients with an active and short disease duration. He suggested that corticosteroids and radiotherapy are only effective during the active phase of fibroblast activation. This is further discussed in paragraph 1.3.

1.5.5 T cell or B cell disease? The question arises whether Graves’ ophthalmopathy is predominantly a B- or a T-cell disease, or whether both pathways contribute. In other words, there is controversy about GO being a Th1 (cell mediated), or Th2 (humoral) autoimmune phenomenon. De Carli et al. found that T cells in orbital connective tissue carry predominantly a Th1 cytokine signature (IL-2 and IFN-γ). In contrast, McLachlan et al. found a trend towards a Th2 cytokine profile, with an IL-4 increase instead of IFN-γ. This was confirmed by Pappa et al., who apart from IL-4 found a mixture of cytokines, such as IFN-γ, IL-10, IL-2 etc with none of them being predominant. All these studies were based on a few patients only, and most of them had been either extensively treated with glucocorticoids or were suffering from longstanding eye disease. Another point of criticism at some of these studies is that proliferation assays of extracted lymphocytes from retroorbital tissue were used, which may not resemble the in vivo situation. Immuno- 

1.5.6 Cytokines and adhesion molecules Retrobulbar fibroblasts may proliferate and produce GAG’s in vitro upon stimulation with a variety of molecules, e.g. IL-1, IFN-γ and TGF-β. These effects can be blocked by antagonists to the respective cytokines, irradiation or corticosteroids. Heufelder et al. showed that ICAM-1 is expressed on cultured orbital fibroblasts upon incubation with various cytokines or with IgG’s purified from serum of Graves’ disease patients. Immunohistochemistry of orbital connective tissue samples from GO patients showed an increased expression of ICAM-1, VCAM-1 and ELAM-1 when compared with tissues from controls. Just a few in vivo studies have demonstrated the presence of several cytokines, such as IFN-γ, TNF-α, IL-1α, IL-1β, IL-2, IL-4, IL-6 and IL-10 in the retro orbital tissue of GO patients. It should be noted that these biopsies were from GO patients who had been extensively treated with corticosteroids and/or radiotherapy and were probably in the inactive fibrotic endstage of their eye disease. Cytokines and other immunomodulatory molecules play a pivotal role in inflammation in autoimmune diseases, and it is very likely that these proteins also play a major role in GO. Therefore it would be very interesting to know which cytokines are present in retroorbital tissues in the early stages of the disease. This might have two important clinical implications. First, a good biological activity marker of GO is lacking, and up to now a surrogate marker of activity, the Clinical Activity Score is used. Secondly, knowledge of which cytokines are crucial in early, active GO might help to select specific immunosuppressive drugs, i.e. anti-cytokine therapy. This issue will be further detailed in Chapters 5 and 9.

1.5.7 Target of the autoimmune attack: fibroblast or muscle cells? There is growing evidence that the orbital fibroblast is the target cell of the autoimmune attack. The grossly enlarged extraocular muscles have drawn investigators’ attention initially to the eye
The focus then changed to the orbital fibroblast. In GO the histological hallmarks consist of retroocular infiltration by inflammatory cells and the accumulation of GAG’s both in the extraocular muscle and the retro orbital connective tissue. The orbital fibroblast does proliferate and produce GAG’s under the influence of certain immunomodulatory molecules, such as cytokines. The cytokine stimulated proliferation of cultured orbital fibroblasts can be inhibited by glucocorticoids. Glucocorticoid treatment is beneficial in active GO, as will be described in paragraph 1.6. Taken together it seems that GO is a disease of primarily the fibroblasts rather than the muscle cells. Figure 6 shows a simplified scheme of the pathogenesis of GO, reproduced with kind permission of the Publisher and Prof. Dr A.P. Weetman.

1.5.8 Autoantigen Many researchers have focussed on finding the autoantigen responsible for GO. This quest has resulted in many proteins which have been put forward as being the sought after golden grail. I will not focus on this quest but only summarize why there is accumulating evidence that autoantibodies against the TSH-R are of pivotal importance in GO, and in Chapter 9 recent developments in the search for the responsible autoantigen will be discussed. The presence of TSH-R mRNA in retroorbital tissue has first been demonstrated by Felicielli et al. Many studies have confirmed these observations and have shown the protein to be present in these tissues as well. TSH-R protein has been found both in orbital fibroblasts of GO patients and, to a lesser extent, in healthy individuals. Recent in vitro studies showed that differentiation of preadipocyte/fibroblasts induces expression of the TSH-R. The expression of TSH-R can be enhanced by adding certain cytokines such as TNF-α and IFN-γ to the culture medium. In some studies the TSH-R was not found or only in GO patients and not in healthy controls. These results may be due to different techniques, or may have to do with a differential expression of the TSH-R in GO patients. In Graves’ thyroid disease autoantibodies against the TSH-R are at the heart of the autoimmune response. Since most patients with GO also suffer from

![Figure 6 Pathogenesis of GO. The extraocular muscles are infiltrated by inflammatory cells, predominantly T cells, thought to be reacting with a thyroid-like autoantigen; at present TSH-R is the prime candidate. Cytokines released by the infiltrate activate fibroblasts to secrete glycosaminoglycans, which trap water and cause muscle swelling. Orbital fat increases when the TSH-R on preadipocyte fibroblasts is stimulated by TSH-R antibodies, increasing the volume of the orbital contents further. (Reproduced with permission)]
Graves’ thyroid disease, and TSH-R autoantibodies are usually present, it seems that it is rather likely that the TSH-R somehow plays a role in GO. This view is supported by the finding of a positive correlation between the CAS and TSH-R antibody levels and also between proptosis and TSH-R antibody levels.\textsuperscript{111,112}

1.5.9 Animal model studies It remains difficult to study autoimmune responses in humans properly, because there is an unknown time lag between initiation and clinical presentation of the disease. Therefore we are confined to the study of experimental animal models. There is no known animal model in which autoimmune Graves’ thyroid disease develops spontaneously. Many efforts with varying degrees of success have been made to induce thyroid autoimmunity in experimental animals, and these have been recently reviewed by Prabhakar \textit{et al.}\textsuperscript{19} Until now, many different animal models have been tried, and all efforts have been dissatisfying. At best they have shown that both Graves’ thyroid disease and GO are very heterogenous disorders. Some features of Graves’ thyroid disease and GO have been induced in mice by TSH-R preparations. In short, AKR/N mice were transfected with the homologous major MHC complex class II molecule and the full length human or murine TSH-R.\textsuperscript{113} Approximately 20% of mice develop thyroid antibodies that stimulate the TSH-R and have increased thyroxine levels, but they do not develop thyroditis. When BALB/c mice and NOD mice are treated with TSH-R primed T cells, the BALB/c mice develop a Th2 response, with production of IL-4 and IL-10, whereas in NOD mice a Th1 type response together with thyrocyte destruction and production of IFN-\(\gamma\) was observed.\textsuperscript{114} The orbits of BALB/c mice, but not NOD mice, showed changes such as infiltration by immune cells, proliferation of adipose tissue and edema in the extraocular muscles in 70% of cases. Based on these results a Th2 autoimmune response to the TSH-R was proposed to be an initiating event in GO.\textsuperscript{114} Similar results were reported after genetic immunisation of outbred mice with TSH-R cDNA.\textsuperscript{115} These could not be reproduced in another laboratory, despite identical experimental conditions.\textsuperscript{116} Taken together with the heterogeneous response of BALB/c and NOD mice to the same stimuli, it is evident that a good and reproducible experimental animal model for GO is still lacking. This might be partly attributable to the lack of environmental stimuli, such as major life events and smoking, that are present in GO patients and cannot be reproduced easily in experimental animal models. Furthermore, the exact sequence of events in GO is not known nor is the responsible autoantigen, which further complicates the search for an animal model.

1.6 Treatment

All GO patients should be urged to quit smoking because, patients who continue smoking benefit less from treatment of their eye disease than patients who quit their habit.\textsuperscript{64,66} Since both hyper- and hypothyroidism have a slight adverse influence on the eye disease, euthyroidism should be restored and maintained.\textsuperscript{117,118} Treatment with radioactive iodine for Graves’ hyperthyroidism can have a deteriorating influence on the eye disease, although often transient in nature: the worsening can be prevented by corticosteroids.\textsuperscript{119}

\textbf{Local measures} Local measures such as artificial tears and lubricating ointments should be liberally prescribed to all GO patients, to prevent keratopathy and to alleviate gritting sensations. Other measures include the use of dark glasses or prismatic correction to reduce double vision.

\textbf{Immunosuppressive treatment modalities} There is sufficient evidence that corticosteroids, both
oral and intravenous are effective, with intravenous pulses of methylprednisolone being superior to oral steroids.\textsuperscript{121-124} Orbital irradiation was found to be equally effective to corticosteroids in a randomised controlled trial.\textsuperscript{121} Since then others have found orbital irradiation to have either beneficial or only marginal effects.\textsuperscript{125-128} Orbital irradiation nor glucocorticoids have a relevant effect on proptosis, so in patients with major proptosis decompressive surgery is often still needed later in treatment. Marcocci et al. demonstrated that 88% of GO patients responded to the combination of methylprednisolone pulses and orbital irradiation.\textsuperscript{126} However, glucocorticoids are associated with considerable side effects such as hypertension, diabetes mellitus and depression.\textsuperscript{121} Recently, four deaths due to liver failure were described after methylprednisolone pulses.\textsuperscript{129,130} Orbital irradiation is associated only with some transient and mild side effects such as local irritation.\textsuperscript{121} The long term safety of orbital irradiation will be addressed in Chapter 7. Some studies have focussed on other immunosuppressive treatment modalities such as cyclosporin\textsuperscript{122,131-133}, somatostatin analogues \textsuperscript{134}, immunoglobulins\textsuperscript{135,136}, plasmapheresis\textsuperscript{137} and pentoxifylline \textsuperscript{138}, all of which were reported to have limited effect.\textsuperscript{139} It is difficult to compare the results of these studies, because only a few were randomized controlled trials and extensively treated patients have been included in some studies.\textsuperscript{126}

**Decompressive surgery** When patients are diagnosed with inactive GO, or their disease has been rendered inactive by immunosuppressive treatment, rehabilitative surgery can be done. In the case of severe proptosis decompressive surgery is performed. The goal of decompressive surgery is to create space for the increased orbital content. In this procedure orbital walls — usually two or three walls depending on the amount of proptosis — are removed. There are various surgical techniques for decompression, all of which lead to the reduction of proptosis, so the preferred operation technique is the one in which the surgeon is most trained.\textsuperscript{29} The only exception being when decompressive surgery is done because of optic neuropathy. When optic neuropathy is present the medial wall of the orbit should be removed to relieve the pressure on the optic nerve. This medial wall is easily accessible via the coronal route and many surgeons prefer this route when optic neuropathy is present, although other techniques are advocated as well.\textsuperscript{29,140,141} Squint and eyelid surgery The next step in the surgical rehabilitation of a GO patient is squint surgery in order to reduce double vision. Finally eye lid surgery can be done to restore the patients appearance.

There is still a need for more effective immunomodulatory therapy with less side effects in GO patients. For an approach that is curative rather than just shortening the duration and dampening the severity of eye disease, we should gain a better insight about the autoimmune process in the orbit, and aim at the reversal of this process. Of course there are costs of therapy to consider, especially in an era in which increasing costs of health care are politically hard to sell. In chapter 9 I will propose management guidelines for GO patients.

### 1.7 Outline of the present thesis

This thesis consists of two parts. In the first part we describe the search for a serum marker in GO patients, which could be used to select patients who might benefit from immunosuppressive treatment. Furthermore, we tried to elucidate the cytokine network in the immunopathogenesis of GO in order to select future, more selective immunomodulatory therapies. The second part of this thesis deals with aspects of medical and surgical management of GO.
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