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

Wakelkamp, I.M.M.J.

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Chapter 9

General discussion
9.1 In search of better markers

The concept of disease activity, which originates from observations on the natural course of GO is attractive because it might explain why one-third of patients do not respond to immunosuppressive therapy, if administered to an otherwise unselected group of consecutive patients. Looking at the curves of Rundle (Chapter 1; Figure 3), one can imagine that only patients in the active stage of the disease will respond to immunosuppressive therapy, while patients with inactive fibrotic disease will not respond. As described in Chapter 1, paragraph 1.3 sometimes the distinction between active and inactive GO is easily made, but more often than not this discrimination is difficult to call. Ideally, only patients with active GO should be selected for immunosuppressive therapy, thereby avoiding the exposure to potential serious side effects among patients who will not benefit from these therapies. In order to select the patients with active GO we have searched for an objective biological marker of GO disease activity. Because cytokines and adhesion molecules are involved in autoimmune responses and some can be readily measured in human serum using ELISA, we have searched amongst these molecules.

Biological markers In Chapter 2 we have demonstrated that serum concentrations of Th1- and Th2-derived cytokines and cytokine receptors were higher in euthyroid untreated GO patients than in healthy controls matched for sex, age and smoking habits. The cytokines found to be increased in GO patients include sIL-2, IL-6, IL-6R, TNF-αRI and II and sCD30, but not IL-1RA. Our results are in accordance with the finding that sIL-2R and IL-6R levels are increased in GO patients, and that sIL-1RA levels are comparable with levels in healthy controls. Hofbauer et al. measured sIL-1RA levels in 27 GO patients and found that sIL-1RA was decreased in 18 smoking patients compared to the levels in 9 non-smoking patients. Increased baseline sIL-1RA levels in their study were associated with a response to radiotherapy. We could not confirm these findings, nor could others. We used the same assay and actually found higher sIL-1RA levels in smokers than in non-smokers. It might be that this discrepancy is due to the larger sample size of our study, or to a different patient selection. We included patients on the basis of disease severity, not activity and consequently many appeared to have had inactive disease whereas Hofbauer et al. included active GO patients with <4 months disease duration. Furthermore, we also measured sIL-1RA in healthy controls who had serum concentrations comparable with GO patients. In chapter 3 we have measured serum IL-18 levels but here we did not observe a difference between GO patients and matched controls. Mysliwiec et al. reported increased serum IL-18 concentrations both in 17 euthyroid Graves' disease patients with GO and in 14 euthyroid Graves' disease patients without GO compared with 12 controls. This contrasting finding might be due to their smaller sample size. In Chapter 4 we have demonstrated that serum sICAM-1, sVCAM-1 and sELAM-1 concentrations were higher in euthyroid GO patients.
compared with healthy matched controls and euthyroid Graves' disease patients without GO. Our results corroborate the results from others who found sICAM-1 levels and sELAM-1 levels to be increased in patients with GO compared with healthy controls or patients with Graves' disease without GO.

Do the increased serum cytokine and adhesion molecule levels reflect autoimmune disease in the orbit or in the thyroid?

It seems unlikely that the thyroid disease alone is responsible for the elevated serum cytokine and adhesion molecule concentrations in GO patients. Patients had been rendered euthyroid with antithyroid drugs for at least 2 months before enrollment in our studies. No correlation was observed between cytokine or adhesion molecule levels and 

\[ \text{FT}_4 \]

levels or parameters of autoimmune thyroid disease such as TPO or TSH receptor antibodies. In addition, patients who suffer from Graves' disease without GO and who are kept euthyroid with block replacement therapy, have similar serum levels of sICAM-1, sVCAM-1 and sELAM-1 as healthy controls. Others have also found that serum levels of several cytokines and adhesion molecules are increased in Graves' disease patients with GO compared with those without. Yet serum levels of IL-6, sICAM-1 and sELAM-1 were higher in all Graves' disease patients than in healthy controls. Therefore, the elevated levels of certain serum cytokines and adhesion molecules might reflect ongoing autoimmune reaction in the orbit.

On the other hand, serum cytokine levels do not correlate with various parameters of Graves' ophthalmopathy, such as disease duration, disease severity, or disease activity measured by the CAS. One exception were sICAM-1 levels which correlated with disease severity as assessed with the Total Eye Score (\( r=0.40, P=0.002 \)), but not with other parameters. Possibly, these findings reflect the fact that the CAS or disease duration are surrogate markers for disease activity. Alternatively, the increased levels of cytokines and adhesion molecules in serum reflect an overall heightened activation of the immune system in Graves' patients. Still another explanation might be that GO patients have a different genetic background compared with controls. Differences in cytokine production by lymphocytes in vitro have been demonstrated in subjects with certain HLA-B8, DR3 haplotypes when compared with others lacking this haplotype.

We have also established that smoking significantly influences serum cytokine levels, for levels of IL-6R and sIL-1RA tend to be higher in smokers. Hofbauer et al., as described before, found lower serum concentrations of sIL-1RA in smokers suggesting that an inability to sufficiently increase IL-1RA levels has a negative influence on the outcome of GO. We could not confirm these findings. We found no change in sIL-1RA levels 6 months after orbital irradiation. Results from other studies are in agreement with our results, in that an association was found between increased levels of IL-1RA in 229 healthy blooddonors and environmental factors such as smoking and non-steroidal anti-inflammatory drugs rather than with genetic or biological factors such as age and gender. Smoking habits did not affect serum IL-18 levels, but influenced serum adhesion molecule levels. We found sICAM-1 and sVCAM-1 levels to be different in smokers compared with non-smokers, both in healthy controls and in GO patients. Most other studies have not taken smoking habits in consideration and this might have influenced their results, because GO patients tend to smoke more often than the general population and smoking is a known and substantial risk factor for GO. The mechanism by which smoking increases serum cytokine and adhesion molecule levels is not known. Bergmann et al. found that healthy smoking
women had higher levels of sICAM-1 than non-smoking women, as well as a higher leucocyte count and —subsequently— more monocytes. Together with the observation that anti heat shock protein 72 antibody levels are higher in smokers than in non-smokers, the above data suggest that smoking leads to inflammation, lymphocyte recruitment, increased adhesion molecule expression and as a result, more cell adhesion to vessel walls.

**Our main quest was to find a good biological marker of activity and did we find one?**

We found that a combination of sCD30, TNF-αRI and IL-6 could predict the response to radiotherapy in a group of 62 GO patients with moderately severe GO. From these patients 55% had a beneficial response to radiotherapy, and presumably their eye disease was in the active stage, so the disease improved by the immunosuppressive treatment. However, a receiver-operator-characteristics curve taking into account IL-6, sCD30 and TNF-αRI yielded an area under the curve of 0.69, indicating that this combination of serum cytokines does not serve as a clinically applicable biological marker for disease activity. Apart from the fact that there is too much overlap in serum values of responding and non-responding patients, the routine determination of these three different cytokines in the serum of GO patients would be expensive.

It is likely that a biological serum marker for GO can better be sought outside the realm of cytokines and adhesion molecules. Although these molecules are abundantly present in inflamed tissues, they only partly leak into the systemic circulation.

**Have others found better markers for disease activity?**

Gerding et al. have found that octreotide scintigraphy could predict response to radiotherapy in 22 GO patients, using a cut-off value for orbital/background uptake ratio of 1.85 resulting in a positive predictive value of 92% and a negative predictive value of 70%. Octreotide scintigraphy is not routinely available, it is also very expensive, impinges a significant radiation dose and its quantitative assessment is technically difficult. Contrast enhanced MRI does not seem to be helpful to predict response to orbital irradiation, as was evident from a study in which 54 GO patients treated with 20 Gy radiotherapy were screened. Although our group found that T₂ relaxation time had a limited positive predictive value of 64% and negative predictive value of 92% in predicting response to radiotherapy. Recently, Martins et al. demonstrated in 127 GO patients that urinary GAG’s and serum hyaluronic acid were independent determinants of the CAS, i.e. in patients with a CAS ≥ 3 the concentration of hyaluronic acid is higher and the urinary GAG excretion is increased compared with patients with a CAS ≤ 2. Terwee et al. found in a complicated prediction model that urinary GAG excretion had some value to predict no change upon radiotherapy. Autoantibodies against the TSH-R have a reasonable direct correlation with activity of GO, measured by the CAS. Also a correlation was found with severity of eye disease, measured by proptosis. This strong association between TBI levels and the CAS was still present after immunosuppressive therapy. With new commercially available assays, TBI concentrations can be demonstrated in virtually all GO patients. But TBI levels were not helpful in predicting response upon radiotherapy. Terwee et al. combined various parameters of disease activity in order to be able to predict therapeutic outcome in GO. They found with multivariate analysis that duration of GO, soft tissue involvement, elevation, serum sIL-2R and sCD30, eye muscle reflectivity on ultrasound and octreotide uptake ratio were significant predictors of a response to orbital radiotherapy in a group of 66 GO patients. Other parameters such as urinary GAG excretion, gender, duration of...
GO, soft tissue involvement, serum IL-6 and eye muscle reflectivity could predict for no change upon radiotherapy. Taken together they were able to predict response in 89% of GO patients. These results should be confirmed, though it is difficult and expensive to routinely measure so many parameters in each patient.

Pending a better alternative, it seems that the combination of a relatively short eye disease duration (arbitrary <18 months) and a high CAS (≥4) together with one of the imaging techniques is the best available instrument to decide whether or not a patient should receive immunosuppressive therapy.

9.2 In search of better management

9.2.1 Cytokines The few available studies concerning the presence of cytokines and the TSH-R in orbital tissues of GO patients consist of samples of extensively treated, and thus presumably inactive patients. We were able to obtain orbital connective tissue samples from untreated GO patients who were in the most active phase of their disease (n=6) and samples from inactive GO patients (n=11), all of whom underwent surgical decompression. We quantitatively compared the presence of mRNAs for various cytokines and the TSH-R in these tissues as described in Chapter 5. The cytokine profile in patients with active GO is dominated by proinflammatory cytokines, with markedly increased levels of IL-1β, IL-6 and IL-8 compared with patients with inactive GO. The inflammatory cytokine IL-10 is also increased in orbits with active GO, and this could imply involvement of T regulatory cells in the early phase of GO. Furthermore, there is a trend towards a predominance of Th1 over Th2-derived cytokines in these patients with active GO as is evident from higher IL-2, IFN-γ and IL-12 expression. Other cytokines such as IL-13, IL-18, IL-1RA and TNF-α had similar levels in active and inactive patients and IL-3, IL-4 and IL-5 were absent in most samples.

Since orbital connective tissue of GO patients contains several cell types, we do not know the precise source of the cytokine mRNAs. Orbital fibroblasts and preadipocytes as well as infiltrating T cells and macrophages or a mixture of these cells may produce these cytokines. Localisation techniques such as FISH might determine the involved cell types and their relative contribution at the mRNA level.

Our results need to be confirmed at the protein level, but since cytokines are usually present in low concentrations it might be difficult to visualise them with immunohistochemistry. Using another approach, Aniszewski et al.24 analysed T-cell clones and found particularly Th1 type T-cell clones in early GO compared with late GO as did Pappa et al.25 using immunohistochemistry. Some of the cytokines we found, such as IL-2 and IL10 have been found by McLachlan et al.26 in 5 well defined GO patients. However, they were not able to detect any IFN-γ mRNA, which might be because their patients had inactive GO. Kumar and Bahn27, with a quantitative approach, found IL-1β, IL-6, IL-8, IL-10, TNF-α and IFN-γ mRNAs to be present in orbital connective tissue of 6 GO patients and 2 controls without history of Graves’ disease at autopsy. The cytokine gene expression was higher in GO patients than in controls and IL-4 and IL-5 mRNA was not found in any of the orbital tissues. However, there results are difficult to interpret because, three patients had been pretreated with corticosteroids for several months and most had longstanding eye disease. In a large study, Hiromatsu et al.28 found mRNA of various cytokines in orbital connective tissue of 29 extensively treated GO patients. As this group of patients was extensively treated with orbital irradiation and corticosteroids it is reasonable to assume that their eye disease was in
the inactive phase. Our results in the inactive patients were in accordance with theirs with the exception of IL-4, that was not present in the orbital connective tissue of our 18 GO patients.

A better understanding of the sequence of events in the orbits of GO patients might be instrumental in delivering more specific immunomodulatory interventions instead of general nonspecific immunosuppressives such as radiotherapy or glucocorticoids.

Based upon our results in active, untreated GO patients drugs that counteract a Th1 or proinflammatory cytokine may have potential. Blocking IL-2 or IFN-γ might be considered in order to antagonise the Th1 effect. We are not aware of clinical studies using monoclonal antibodies interfering with the IFN-γ receptor. In contrast, many studies have focussed on blocking the effect of the IL-2 receptor. IL-2 is only produced by activated T cells, and serves to activate numerous other key cells in the immune system, such as T helper cells, B lymphocytes, macrophages and natural killer cells. Anti-IL-2 receptor antibody therapy has applications both as anti-tumour therapy in HTLV-1 induced adult T cell lymphoma and in autoimmune diseases such as uveitis or psoriasis. Another approach could involve IL-1RA administration, which by competition for the IL-1R blocks the IL-1 effect and thereby a major route in the inflammatory pathway. A further mechanism by which blocking IL-1 might hugely impact the autoimmune response in GO is that activation of cultured fibroblasts from healthy donors can be achieved through IL-1β stimulation. IL-1RA is already being used in treating rheumatoid arthritis, just like anti-TNF-α, and the tolerance seems to be comparable to that of anti-TNF-α. A restriction in the application of these anticytokine therapies is an increased risk of infections such as tuberculosis. Based upon our results in active, untreated GO patients it seems that anticytokine therapy such as anti-TNF-α, which is currently used in Crohn's disease and rheumatoid arthritis, is not a good choice. Recently, Paridaens et al. in a pilot study showed that 12 weeks of treatment with etanercept, an anti-TNF drug, had a slight favourable effect in 10 GO patients with mild to moderately severe, shortstanding eye disease. Prospective clinical trials in GO patients with any of these specific immunomodulatory drugs are to be awaited.

9.2.2 T cells GO seems to be the result of a T cell mediated disease. Autoantibodies do probably play a role in the pathogenesis, but whether they perpetuate or initiate the autoimmune response is not clear. An argument against a mechanism that involves initial B-cell activation and antibody production is that there has never been a report of a baby suffering from GO after being born to a mother who suffered from Graves' disease. Since newborns can suffer from thyrotoxicoses via placental transport of TSH-R antibodies, inducing hyperthyroidism and goiter in the infant, GO would have been expected if these antibodies are the primary causative agents. Gene polymorphisms such as CTLA-4 and HLA classes that interfere with T cell function, might be at the core of the susceptibility to GO. CTLA-4 is expressed on the surface of activated T cells and plays together with another costimulatory molecule CD28, a critical role in the T-cell response to antigen presentation. T cell activation is initiated when the antigen-specific cell surface T-cell receptor (TCR) engages the antigen, which is bound to an MHC class II molecule on the surface of an antigen-presenting cell (Figure 1). A second, costimulatory signal is required to complete this activation which then leads to T-cell proliferation and cytokine production. In the absence of a positive co-stimulatory signal, the antigen-TCR is ineffective, and causes the T cell to be refractory to further stimuli or induces apoptosis of the cell. This positive co-stimu-
latory signal is provided by the interaction of CD28 and its ligand B7.1 (CD80) and B7.2 (CD86) on antigen-presenting cells. CTLA-4 also binds to B7 ligands, with greater affinity, but offers inhibitory signals to T-cell activation. It would be interesting to enhance CTLA-4 availability to inhibit T-cell activation. Recently, CTLA-4 immunoglobulins have been used in phase 2 trials in patients undergoing renal transplantation and preliminary results show the effect to be similar to cyclosporin in preventing rejection. May et al. very recently demonstrated a screening model for the testing of anti-costimulatory monoclonal antibodies in a SCID model.

Another approach could include the use of altered peptide ligands, defined as peptides in which amino acid replacements cause a different activation of the T-cell, as a result of the modified interaction with the TCR. Designed modified peptides for T cell mediated immunotherapy could be the future, although recent results are rather disappointing.

Another way of interfering with T-cell function would be the use of somatostatin analogs. The rationale to use somatostatin analogs for GO is that octreotide scintigraphy is often positive in active GO patients, meaning that octreotide receptors must be present. Many cell types, among them lymphocytes and fibroblasts express somatostatin receptors. In addition, fibroblast proliferation can be inhibited by adding somatostatin analogs to the culture medium and functional somatostatin receptors are present on orbital fibroblasts. Somatostatin analogs might act through different mechanisms, including inhibition of cytokine secretion and direct interaction with somatostatin receptors on lymphocytes. Beneficial responses were reported in small, uncontrolled, non-randomised studies, but a recent randomized controlled study in 51 patients with mild, active GO demonstrated no effect on GO activity and a very small effect on proptosis, which was statistically significant but probably not very relevant clinically, because the proptosis difference was about 1 mm. It could be that novel somatostatin analogs such as SOM230, is effective in GO, because this compound shows a higher affinity for somatostatin receptor-1, -3 and -5, and a slightly lower affinity for somatostatin receptor-2 than octreotide and lanreotide. Somatostatin analogs might act through different mechanisms, including inhibition of cytokine secretion and direct interaction with somatostatin receptors on lymphocytes. Beneficial responses were reported in small, uncontrolled, non-randomised studies, but a recent randomized controlled study in 51 patients with mild, active GO demonstrated no effect on GO activity and a very small effect on proptosis, which was statistically significant but probably not very relevant clinically, because the proptosis difference was about 1 mm. It could be that novel somatostatin analogs such as SOM230, is effective in GO, because this compound shows a higher affinity for somatostatin receptor-1, -3 and -5, and a slightly lower affinity for somatostatin receptor-2 than octreotide and lanreotide. Somatostatin analogs might act through different mechanisms, including inhibition of cytokine secretion and direct interaction with somatostatin receptors on lymphocytes. Beneficial responses were reported in small, uncontrolled, non-randomised studies, but a recent randomized controlled study in 51 patients with mild, active GO demonstrated no effect on GO activity and a very small effect on proptosis, which was statistically significant but probably not very relevant clinically, because the proptosis difference was about 1 mm. It could be that novel somatostatin analogs such as SOM230, is effective in GO, because this compound shows a higher affinity for somatostatin receptor-1, -3 and -5, and a slightly lower affinity for somatostatin receptor-2 than octreotide and lanreotide. Somatostatin analogs might act through different mechanisms, including inhibition of cytokine secretion and direct interaction with somatostatin receptors on lymphocytes. Beneficial responses were reported in small, uncontrolled, non-randomised studies, but a recent randomized controlled study in 51 patients with mild, active GO demonstrated no effect on GO activity and a very small effect on proptosis, which was statistically significant but probably not very relevant clinically, because the proptosis difference was about 1 mm. It could be that novel somatostatin analogs such as SOM230, is effective in GO, because this compound shows a higher affinity for somatostatin receptor-1, -3 and -5, and a slightly lower affinity for somatostatin receptor-2 than octreotide and lanreotide. Somatostatin analogs might act through different mechanisms, including inhibition of cytokine secretion and direct interaction with somatostatin receptors on lymphocytes. Beneficial responses were reported in small, uncontrolled, non-randomised studies, but a recent randomized controlled study in 51 patients with mild, active GO demonstrated no effect on GO activity and a very small effect on proptosis, which was statistically significant but probably not very relevant clinically, because the proptosis difference was about 1 mm. It could be that novel somatostatin analogs such as SOM230, is effective in GO, because this compound shows a higher affinity for somatostatin receptor-1, -3 and -5, and a slightly lower affinity for somatostatin receptor-2 than octreotide and lanreotide.

9.2.3 Autoantigen The inactivation of the orbital fibroblasts could perhaps also be achieved by counteracting the responsible autoantigen in GO. In Chapter 5 we have demonstrated that TSH-R mRNA is present more often in orbital fat/connective tissue of patients with active GO than in patients with inactive GO (83% vs. 18%). The presence of TSH-R mRNA was first demonstrated in orbital tissue of healthy controls and GO patients in 1993. Later, both protein and mRNA were confirmed to be contained in orbital fibroblasts of patients
with GO as well as healthy controls, although the immunoreactivity appeared less abundant in the controls.44-46 TSH-R presence was found to be functional.47 Others who found TSH-R expression in GO patients were not able to demonstrate the protein in healthy controls.48 These differences might be explained by the differential sensitivity of the techniques used—RT-PCR, in situ hybridisation and immunohistochemistry—or by differences in expression regulation. Our finding that TSH-R mRNA is predominantly found in active GO would agree with a recent finding that only a subset of orbital fibroblasts express the TSH-R, namely the preadipocytes during their differentiation into adipocytes.49 This finding suggests that the TSH-R is not continuously present in the human orbit and that the expression is induced in the active autoimmune stage of GO. It also explains why some authors have found the TSH-R to be present in orbital tissues of controls and others have not.49 But how important is the presence of the TSH-R on orbital fibroblasts in respect of the autoimmune attack in GO? Since antibodies against the TSH-R are the hallmark of Graves’ disease it is not likely that the presence of the TSH-R in the human orbit is coincidental. The correlation of GO activity and titers of TSH-R antibodies further implies a certain connection.21,22 Also studies performed in experimental animals suggest that an autoimmune response against the TSH-R is contributing to the eye disease.50 So, if the TSH-R is the autoantigen in GO, efforts should be made to block the effects. Perhaps in the future this will be possible with new exciting techniques such as RNA interference (RNAi), a method to dampen or shut of the expression of individual genes using small RNA's.51 Another route might be the use of TSH-R monoclonal antibodies that block the signalling pathway via the TSH-R or that bind to circulating autoantibodies (e.g. cleavable soluble TSH-R).

Evidence has been found for binding of Insuline-like Growth Factor 1 (IGF-1) to the surface of orbital fibroblasts.52 Recently, support for a role of the IGF-1 Receptor pathway in GO was found by Smith et al. who showed that IGF-1 and IgG’s apparently directed against the IGF-1R, from patients with Graves’ disease induce hyaluronan synthesis in cultured orbital fibroblasts of GO patients.53 Upon stimulation of the IGF-1R fibroblasts from Graves’ patients express high levels of IL-16 and RANTES.54,55 Further studies to enlighten the role of the IGF-1 receptor in the pathogenesis of GO are necessary. All in all, aside from the established presence of the TSH-R, many candidate autoantigens have been suggested to play a role in GO. Most of this work has focused on the eye-muscle cell and its surface membrane as the principle target for immune recognition.55 Wall et al. used preparations of porcine eye muscle membrane and first showed that serum from patients with active GO reacted with this preparation. Later they identified a 64 kDa protein followed by a 67 kDa protein, after that a protein between 63 and 67 kDa and later this became a 220 kDa protein. Subsequently, these proteins lost their hoped for disease specificity.55 Future experimental therapeutic interventions should preferably intervene in the early inflammatory phase, by interference with major cytokine routes, T cell activation, or the binding of the responsible autoantigen (i.e. the TSH-R) to its receptor (Table 1).

9.3 Management of GO

All patients should be urgently and if necessary repeatedly advised to quit smoking. When thought to be helpful, nicotine plasters or medication such as bupropion can be applied. The deteriorating influence of smoking on the development and course of GO is described in Chapter 1, paragraph 4.
Table 1 Shows cytokines, TSH-R and CTLA-4 presence in orbital tissue of active vs. inactive patients and possible interactions with specific immunomodulatory drugs.

<table>
<thead>
<tr>
<th>molecule</th>
<th>Present in active, compared with inactive GO?</th>
<th>Antibody available</th>
<th>Effective in other autoimmune diseases?</th>
<th>Potentially useful in GO?</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>↑</td>
<td>yes</td>
<td>+ psoriasis, uveitis</td>
<td>yes</td>
</tr>
<tr>
<td>IL-1RA</td>
<td>=</td>
<td>yes</td>
<td>+ rheumatoid arthritis</td>
<td>yes</td>
</tr>
<tr>
<td>IL-1β</td>
<td>↑</td>
<td>yes, IL-1RA</td>
<td>+ rheumatoid arthritis</td>
<td>yes</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>↑/=</td>
<td>no</td>
<td>+ Crohn’s</td>
<td>yes</td>
</tr>
<tr>
<td>IL-6</td>
<td>↑</td>
<td>no</td>
<td></td>
<td>yes</td>
</tr>
<tr>
<td>IL-8</td>
<td>↑</td>
<td>no</td>
<td></td>
<td>yes</td>
</tr>
<tr>
<td>IL-10</td>
<td>↑</td>
<td>yes</td>
<td></td>
<td>yes</td>
</tr>
<tr>
<td>IL-12</td>
<td>↑/=</td>
<td>no</td>
<td></td>
<td>yes</td>
</tr>
<tr>
<td>IL-13</td>
<td>=</td>
<td>no</td>
<td></td>
<td>no</td>
</tr>
<tr>
<td>IL-18</td>
<td>=</td>
<td>no</td>
<td></td>
<td>no</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-</td>
<td>yes</td>
<td>+ Crohn’s/rheumatoid arthritis</td>
<td>no</td>
</tr>
<tr>
<td>TSH-R</td>
<td>↑</td>
<td>no</td>
<td></td>
<td>yes</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>?</td>
<td>yes</td>
<td></td>
<td>yes</td>
</tr>
</tbody>
</table>

Thyroid treatment

As a first measure, the dysthyroidism that is usually present should be treated, because both hyper- and hypothyroidism have a negative influence on GO and antithyroid treatment has been shown to improve the eye disease. The negative influence of dysthyroidism is likely the consequence of further TSH-R activation on the orbital fibroblasts via stimulation by TSH-R antibodies in hyperthyroidism, and by TSH itself in hypothyroidism.

Treatment of hyperthyroidism can be achieved with antithyroid drugs, such as methimazole or propylthiouracil. Antithyroid drugs can be titrated until normal thyroid hormone levels in the plasma are reached, or alternatively so called block and replacement therapy can be given. In that case a total blocking dose of antithyroid drugs is given, usually 30 mg of methimazole or 450 mg of propylthiouracil in combination with adequate thyroxine substitution. The TSH levels can remain suppressed during a long time, thus it is reasonable to attempt for high normal levels of free T₄. Radioactive Iodine can induce new GO or worsen preexisting GO, but this can largely be prevented by glucocorticoid treatment. Risk factors for worsening of GO upon ¹³¹I, are the degree of hyperthyroidism, i.e. initial T3 > 5nmol/l, high titers of TBI antibodies, smoking and the presence of GO. In patients with active GO, i.e. having a Clinical Activity Score >3, especially combined with a short disease duration, ¹³¹I should be given cautiously combined with prednisone. We usually treat patients during two weeks with 30 mg of prednisone followed by a tapering dose of 5 mg weekly,
in total 8 weeks. Once the active eye disease has reached the inactive stage, $^{131}$I treatment can be given without prednisone. In our 15 patients with optic neuropathy and active GO we continued block replacement therapy and stopped it after completion of all eye treatments some years later. Even though many patients again developed hyperthyroidism and where then treated with radioactive iodine without glucocorticoids, not one of them had a flare-up of GO (unpublished observations). So it seems that when ophthalmopathy is really in the fibrotic endstage, no reactivation of eye disease occurs usually.

Another option to treat hyperthyroidism is to perform a subtotal thyroidectomy or total thyroidectomy, which does not seem to have a negative influence on GO and theoretically can be of benefit because it involves the removal of large amounts of autoantigens.67

Primary hypothyroidism, or hypothyroidism after radioactive iodine or thyroidectomy should be rapidly and adequately suppletted with thyroxine.

**IMMUNOSUPPRESSIVE TREATMENT OF GO**

When the eye disease is active, i.e. a Clinical Activity Score of > 3 is present, combined with a relatively short disease duration of < 18 months, immunosuppressive treatment can be applied. The question then is which immunosuppressive treatment modality is appropriate. In Figure 9.2 we propose a treatment scheme for GO.

In case of optic neuropathy (by definition active eye disease), methylprednisolone pulses are the preferred choice. In Chapter 6 we have demonstrated that patients with optic neuropathy and very active GO can be effectively treated with intravenous methylprednisolone pulses or orbital surgical decompression. However, the primary response to i.v. pulses was better and it seemed that patients who were operated upon needed more treatments than patients who were initially treated with glucocorticoids, although the groups were small. In other words, it seems reasonable to start with methylprednisolone pulse therapy in patients with optic neuropathy, and when there is insufficient improvement or deterioration of visual functions decompressive surgery should be performed without delay. Using this regimen, most patients will eventually regain a good visual acuity.

When patients suffer from active and moderately severe GO, it can be useful, to shorten

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**Diagram:**

**Diagnosis of Graves' Ophthalmopathy**

<table>
<thead>
<tr>
<th>Smoking</th>
<th>Thyroid</th>
<th>Eye</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>if YES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quit smoking</td>
<td>Restore &amp; maintain euthyroidism</td>
<td>Relieve symptoms</td>
</tr>
<tr>
<td>Bupropion</td>
<td>Carbimazole</td>
<td>Sunglasses</td>
</tr>
<tr>
<td>Nicotine patch</td>
<td>Thyroxine</td>
<td>Artificial tears</td>
</tr>
<tr>
<td>Behavioural therapy</td>
<td>Surgery $^{131}$I</td>
<td>Gels</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prisms</td>
</tr>
</tbody>
</table>

**Figure 2A:** General treatment measures for GO patients. $^{131}$I should preferably be applied in inactive patients or in combination with prednisone.
disease duration and prevent further worsening, to apply immunosuppressive therapy. Radiotherapy has a favourable effect on retrobulbar pain, swelling of the eye lids and eye muscle motility. Radiotherapy does not work immediately, the lag time is usual 6 months, and when an immediate effect is aimed at, glucocorticoids are preferred above orbital irradiation. In recent years is has become clear from two placebo controlled RCT’s that radiotherapy has only a mild beneficial effect on GO. Gorman et al. claim that radiotherapy has no beneficial effect whatsoever, but they treated selected patients already extensively treated with glucocorticoids in whom a response is less likely to occur. The design of this trial was peculiar with one orbit irradiated and the other one functioning as control, while trafficking of lymphocytes is not taken into consideration. The combination of orbital irradiation and glucocorticoids seems to have the best

![Diagram](image_url)

**Figure 2B:** Proposes a flow chart for the treatment of Graves' ophthalmopathy.
result with a response in 88% of patients. The obvious reason why orbital irradiation is an elegant therapy is that there are almost no immediate side effects, compared with oral glucocorticoids. But what about long-term effects? Theoretically, orbital radiotherapy may lead to cataract formation, retinopathy and tumour induction. We have demonstrated in Chapter 7 in a cohort of 245 GO patients treated with orbital irradiation and/or corticosteroids, that orbital irradiation is quite safe after a follow-up time of mean 11 years (SD 3). Mortality was similar in irradiated patients (27/159, 17%) compared with non-irradiated patients (10/86, 12%; P=0.264). In our study, as well as in others evaluating a total of 810 patients, no cases of radiation-induced cancer were detected. We, nor others, noticed an increased cataract formation in irradiated patients compared with patients treated with steroids only. We did find a rather high prevalence of cataract of 29% in the irradiated group and 34% in the non-irradiated group. This cataract prevalence was higher than the 10% found by Marcocci et al. which may be explained by the rather sensitive LOCS II criteria we used. Interestingly, we found that patients treated with oral prednisone had a high frequency of posterior subcapsular cataract (22 from 157 patients, 14%), typical for steroid use, compared with epidemiological studies in the general population which showed a frequency of posterior subcapsular cataract of only 4%. Our main question was whether irradiation induced retinopathy. Fortunately, there was no increased prevalence of proliferative retinopathy, the only exception being that patients with diabetes when irradiated for their GO have a 20 fold increased risk to develop retinopathy. We did observe retinal changes, usually one microaneurysm, more often in irradiated patients (21% vs. 2%, P =0.002) than in non-irradiated patients. These small retinal changes have also been found in large population-based studies in healthy individuals with a prevalence between 5 and 10%. The biological relevance of these retinal changes is unclear, but probably minor. Our results are in line with Marcocci et al. who observed retinopathy only in patients with diabetes or hypertension.

Some recent studies showed a similar beneficial effect with a much lower dose of irradiation, namely 2.4 Gy vs. 16 Gy in 86 GO patients. If with a much lower dose of irradiation the same effect can be achieved, this is very attractive because even lesser side effects are to be expected. Orbital irradiation should only be used in non-diabetic patients, and yields the best effects on swelling of the eye lids, retrobulbar pain and eye muscle motility. Glucocorticoids, both oral or intravenous, have a positive effect on the eye disease activity and motility, but at the expense of more side effects. Intravenous methylprednisolone has less side effects than oral corticosteroids, but four deaths due to liver failure have been described. Three out of seven patients from the Pisa group that developed acute liver failure died, and four patients recovered. In two patients there was evidence of steatosis hepatitis on ultrasound, one of them died, and in one patient who recovered there was evidence of reactivation of CMV.

In more severe disease steroids are more powerful and more rapidly effective than radiotherapy, and should therefore be first choice. We prefer methylprednisolone pulses in a lower dose than for patients with optic neuropathy in a weekly schedule of 500 mg i.v. during 6 weeks followed by 6 weeks of 250 mg i.v. (G. Kahaly, abstract American Thyroid Association 2002) We recommend to make an hepatic ultrasound and determine liverenzymes before and during therapy with methylprednisolone. When methylprednisolone i.v. is contraindicated oral steroids can be given with a starting dose of 60 mg daily during two weeks and than a tapering dose with a total duration of 16 weeks.
Other immunosuppressive therapies such as octreotide, plasmapheresis, cyclosporine and immunoglobulins are not discussed here. Most are less effective than glucocorticoids or orbital irradiation.

**Surgical treatment**
When GO is inactive, either by the passing of time or by immunosuppressive therapy there often is residual disease. If there is proptosis, decompressive surgery to reduce proptosis and restore appearance of the patient is a first step. If necessary squint surgery can later be performed to relieve diplopia. The last step in restoring the patients appearance is eye lid surgery.

**Quality of life**
Little is known about the long term effects of GO on health-related quality of life (HRQL) after the eye treatment is considered to be finished. Patients who participated in the follow up study on the long term safety of orbital irradiation were also asked to participate in a study to evaluate the long term effects of GO on HRQL. The results are described in Chapter 8. It is rather striking that so many years later (mean follow-up 11 ± 3years), GO patients still experience marked limitations in physical and mental functions. Therefore our efforts should be directed not only at restoring visual function and appearance but also at coping with limitations in visual functions and psychosocial functioning.

**Concluding remarks**

The last decades we have learned much about the immunopathogenesis of GO. This knowledge has not substantially changed our therapeutie arsenal. The mainstay of GO treatment still consists of immunosuppressive therapy, both glucocorticoids and radiotherapy, and rehabilitative surgery. There has been progress though. Intravenous methylprednisolone gives less side effects than oral steroids and even though 20 Gy of orbital irradiation seems to be safe, the same effect can probably be reached with a much lower dose. Surgical techniques have been improved. But we still have patients with a reduced quality of life due to their eye disease. So future research should be directed to improvement of quality of life, by improving both functional and cosmetical outcome of Graves’ ophthalmopathy. Perhaps with new immunomodulatory drugs better results with less side effects can be reached. Efforts should be made to prevent Graves’ ophthalmopathy by discouraging smoking in the population and possibly by early diagnosis and treatment of associated Graves’ hyperthyroidism. In patients with already present disease efforts are required to prevent worsening of eye disease.

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