This thesis describes the outcome -survival- of a large group of 186 consecutive patients with chronic active hepatitis of various etiologies, and describes in detail the progress of 21 patients from this group with 'autoimmune' chronic active hepatitis maintained on standardized immunosuppressive therapy.

Idiopathic 'autoimmune' chronic active hepatitis (IAI-CAH) is generally considered as a primarily hepatocytic liver disease of unknown cause, characterized by (episodes of) periportal and portal inflammation and piecemeal necrosis, with the potential to progress to cirrhosis or associated with cirrhosis. It lasts 6 months or longer.

The introduction briefly reviews the current knowledge on IAI-CAH. This is put into a historical perspective, definitions are summarized, clinical symptoms and differential diagnoses are mentioned, data on dismal prognosis in untreated disease and improved prognosis in treated IAI-CAH are reviewed. Treatment options are summarized, problems in the diagnosis and management of the disease are mentioned, and an overview regarding parameters of inflammation and liver function useful in monitoring IAI-CAH is provided. Furthermore, the present knowledge on pathogenesis and possible aetiologies of the disease is put in an immunological context. Untreated IAI-CAH has a high morbidity and mortality. Early mortality is typically due to liver failure, while late mortality usually results from the complications related to cirrhosis. Immunosuppressive therapy can reduce inflammation and enhance survival. Formation of cirrhosis can continue despite induction of 'remission' from CAH. Clinical, histological and biochemical findings must all be considered in concert for decisions concerning therapy. Data are scarce on early changes in inflammation and liver function during immunosuppressive therapy. No optimal tests for the follow-up of IAI-CAH are available. Histology forms the basis for the diagnosis of CAH, but may be insufficient and impractical for follow-up once therapy has been started. Standard laboratory blood-tests do not accurately reflect morphology. Until now, limited information was available regarding changes in hepatic functional capacity during immunosuppressive therapy of IAI-CAH. This warranted a search into parameters of activity and hepatic functional capacity in IAI-CAH.

Section 1 deals with patients and methods.

Chapter 1 provides data on the patients included in these studies.
Chapter 2 refers to the methods used in the studies.

In Chapter 3, we compared numerical histological scoring of activity with conventional pathological descriptions of treated and untreated CAH. The proposed histological activity score (HAS) correlated very well with conventional scores. In contrast to the original method of scoring, we propose to leave fibrosis/cirrhosis out of this score of
activity. Maximum HAS is 18. HAS≤1 denotes the (virtual) absence of inflammatory infiltrate. A HAS of 2 or 3 usually denotes a histology similar to chronic persistent hepatitis (CPH); 90% of biopsies with a histology similar to CPH when scored conventionally, and 36% of biopsies with minimal CAH (CAHmin.), showed a HAS≤3. We therefore termed 1<HAS≤3 "partial histological remission" and propose to reserve the term "complete histological remission" for HAS≤1. We conclude that HAS accurately reflected histological activity of CAH, and is more accurate than conventional descriptions for serial measurement of morphological activity of disease. Furthermore, it enables comparison with other parameters.

In Chapter 4, three radioimmunoassays for the determination of the serum N-terminal propeptide of collagen type III were compared in chronic active hepatitis: a new rapid equilibrium type of assay based on the human propeptide, developed by Risteli and Risteli, was compared with the PIIINP RIA-gnost® (Behring), and the 'PIIINP Fab assay®'(Behring), both based on a reference bovine PIIINP antigen (col 1-3) and antibodies raised in rabbits. The correlation between the human PIIINP RIA and the PIIINP RIA-gnost® was excellent, with less correlation with the PIIINP Fab assay®. The new human assay is therefore as accurate as the standard PIIINP RIA-gnost® used thus far, but it is not sensitive to smaller degradation products. Therefore, unlike in the PIIINP RIA-gnost® (Behring), no serial dilutions of the sera and no 50% intercept method for calculating the results are necessary in this new PIIINP assay, since the standards and the serum samples give parallel inhibition curves. This solves the most important problems that have been inherent in the determination of PIIINP, and provides a rapid reliable test. We used this tests in studies on PIIINP that will follow.

In Chapter 5, the establishment of a modified radioimmunoassay for the determination of autoantibodies against 'liver-specific membrane lipoprotein (LSP)' is described. This modification of the original procedure yielded a rapid, reproducible and quantitative assay for determination of anti-LSP antibodies.

In section 2 Chapter 6, we performed an analysis of survival of all 186 consecutive patients with CAH of various aetiologies included in the current study. 85(±3) percent of all 186 patients with CAH survived 5 years, 70(±4) percent survived 10 years, 55%(±5) survived 15 years, and 40%(±10) survived 20 years after diagnosis. Ages between therapy groups and aetiological groups did not differ significantly. Cox's proportional hazards estimation identified increasing age, presence of antiHBs, and no therapy (patients receiving standard immunosuppression had the best prognosis) as independant risk factors. Sex, presence or absence of antinuclear antibodies, anti-smooth muscle antibodies, level of serum alanine aminotransferase or serum cholinesterase did not influence survival. Most causes of deaths registered in patients with CAH were not directly related to the liver disease. Remarkably, there was a high incidence of plasmacytoma.
In Section 3, several parameters of inflammation and liver function, their relations, and the changes during immunosuppressive therapy are evaluated in 21 patients with 'autoimmune' CAH.

In Chapter 7, routine liver biochemistry in IAI-CAH before and during standardized immunosuppression was serially analyzed. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gammaglobulin (GG) were elevated at the start and improved considerably to normal median values with therapy. However, ALT and AST remained elevated between one and three times the upper reference limit in about half of the patients. Conjugated bilirubin was elevated, then normalized within two months to normal values in all patients. Median unconjugated bilirubin was normal at the start, nevertheless values declined within the first two months of therapy.

Chapter 8 describes histology and its changes during immunosuppressive therapy (0, 2, 14 and 26 months). The high median HAS declined considerably within the first two months of therapy, with further improvement during the next two years of therapy. At two months 67 percent of the patients had already improved to a HAS≤3. The fixed-dose therapy schedule was able to improve histology in 95% of the patients. A HAS≤1 ('complete histological remission') was achieved in 80 percent of the patients and 1<HAS≤3 ('partial histological remission') in 10 percent of the patients within two years of treatment. Periportal and lobular inflammation had disappeared in all of these patients in remission. Continuing decrease in portal and periportal inflammatory activity, with disappearance of plasma cells and decrease in numbers of lymphocytes, was observed during the two years of immunosuppressive therapy. Usually, after two years only lymphocytes remained. HFS was high (median score 3) and did not change during therapy.

Chapter 9 shows that hepatic protein synthesizing capacity, impaired in the majority of the 21 AI-CAH patients, normalized in the course of two years in all patients under investigation, despite the presence of cirrhosis in all but one patient. This was reflected in increasing and normalizing serum albumin (ALB), (pseudo-)cholinesterase (CHE), plasma antithrombin III (AT III), and decreasing and normalizing activated partial thromboplastin time (APTT), usually already in the first 14 months of therapy. The median prothrombin time (PT) was slightly elevated but did not change during therapy, although there was a trend to a transient further increase into the abnormal range within the first four weeks of therapy. CHE, unlike ALB and AT III was still increasing within the second year of therapy. Hence, CHE may be the most sensitive of the parameters of hepatic protein synthesizing capacity under investigation.

Chapter 10 shows that the acute-phase response is depressed in CAH: serum values of C-reactive protein (CRP) are only slightly elevated during this inflammation of its producing organ, and these levels still correlated to periportal inflammation scores. In
contrast to CRP, serum amyloid precursor A protein (SAA) levels are normal in CAH, and slightly increase during therapy. SAA values are related to CHE: its levels reflect hepatic protein synthesizing capacity and not inflammation in CAH.

Chapter 11 describes that the serum N-terminal propeptide of collagen type III (PIIINP) is elevated in 'autoimmune' CAH. PIIINP values rapidly decrease in the first two months of therapy. PIIINP levels finally normalize, or nearly normalize, but this can take 14 months of therapy despite a more rapid improvement in histology. This delay in normalization, and the incompleteness of normalization in half of the patients, may clarify why cirrhosis can develop during immunosuppressive therapy. PIIINP is predominantly correlated to histological inflammation and to ALT, but its levels also show a weak inverse correlation to CHE, probably reflecting reduced degradation of PIIINP in the liver during severely impaired liver function.

In Chapter 12, serum ALT and PIIINP were studied in 16 CAH relapses occurring during therapy withdrawal in 9 patients. During remission, ALT was below twice the upper reference limit, and PIIINP was below the reference limit in all cases. Using two or three times the upper reference limit of ALT as 'cut-off' for diagnosis of CAH-relapse, as recommended in the literature, ALT only rose above this limit median one week before the peak-ALT. An increase of PIIINP above the reference limit occurred weeks to months before the peak-ALT was reached. Using ALT, it was only possible to detect relapse as early as with PIIINP if each patient served as his/her own control for ALT, with the (arbitrarily chosen) upper reference limit at 1.25 times the ALT value obtained during histological remission (HAS≤3 and preferably HAS≤1).

Section 4 describes some immunological abnormalities in chronic active hepatitis, and their clinical relevance. The results in Chapter 13 show that autoantibodies to 'liver-specific membrane lipoprotein (LSP) were invariably present in patients with 'autoimmune' CAH requiring standardized immunosuppressive therapy. Titres declined in all patients during such therapy. In the course of two years anti-LSP became undetectable in half of the patients, while it remained detectable in the other half. As shown by others, presence of anti-LSP probably indicates ongoing stimulation of B-lymphocytes as the result of a defect in inducers of LSP-specific T-suppressor lymphocytes.

In Chapter 14, serum anti-LSP antibodies in 12 CAH relapses occurring during therapy withdrawal in 9 patients were studied. At the start of therapy withdrawal, all patients were in histological remission, and in only one case anti-LSP was detectable. Weeks to months before the peak-ALT was reached, anti-LSP became detectable where previously undetectable (with one exception). A change from undetectable to detectable anti-LSP preceded relapse, and it preceded its detection by the conventional serological definition of relapse (ALT>3x the upper reference limit). It also preceded "non-
remission" based on ALT exceeding two times the upper reference limit. This supports the theory that in IAI-CAH anti-LSP should be undetectable before considering to withdraw immunosuppressive therapy.

Chapter 15 describes that autoantibodies to a soluble liver antigen (SLA) are present in about 10 percent of our population of patients with idiopathic 'autoimmune' CAH. No patients with anti liver-kidney-microsomal antibodies type 1 (anti-LKMI) -denoting a more severe type of IAI-CAH- were detected in this population. Therefore, our patients with IAI could be divided into three of the recently proposed four subtypes of IAI-CAH with the help of ANA, SMA and SLA. Survival in these three groups did not differ, and presence or absence of the antibodies mentioned were not independent prognostic factors. This questions the clinical significance of this classification of IAI-CAH in four subtypes. However, the groups may have different aetiologies of IAI-CAH.

Chapter 16 shows that anti-cardiolipin antibodies (ACA), as detected by ELISA, were not infrequent in CAH and 'CAH-related disorders' of various aetiologies, with the highest prevalence and titres in IAI-CAH. In contrast, anti-DNA antibodies, detected by immunofluorescence with the kinetoplast of Crithidia Luciliae as a substrate, were absent in 98 percent of CAH patients, allowing the possibility of an immunological distinction from SLE. The prevalence of anti-DNA measured by ELISA was higher than with the immunofluorescence mentioned, especially for anti-DNA antibodies of the IgM class. This difference may be due to a difference in avidity of the anti-DNA antibodies measured using both methods. From the results, it also followed that cross-reactivity between ACA and anti-DNA was limited in CAH and, if present, probably only applies to anti-DNA of low avidity.

Chapter 17 shows the high incidence and titres of IgG-class antibodies to cytomegalovirus-induced late antigens (CMV-LA) in IAI-CAH and HBsAg-positive CAH. Frequently, anti-CMV-LA and anti-LSP were both present in IAI-CAH. However, there was no significant correlation between presence of these antibodies. Binding of anti-LSP in the radioimmunoassay for anti-LSP was not inhibited by addition of CMV-LA. Antibodies to the defined CMV-induced antigens 'immediate early antigen (IEA)' of 67 kD and to 'membrane antigen (MA)' of 58 kD molecular weight were only positive in patients with anti-CMV-LA IgG, not in patients with anti-LSP but without anti-CMV-LA IgG. From these results, it can be deduced that an aetiological role of CMV in IAI-CAH by molecular mimicry between CMV-induced membrane antigens and LSP is highly unlikely. This does not exclude an aetiological role for CMV by means of other pathways.