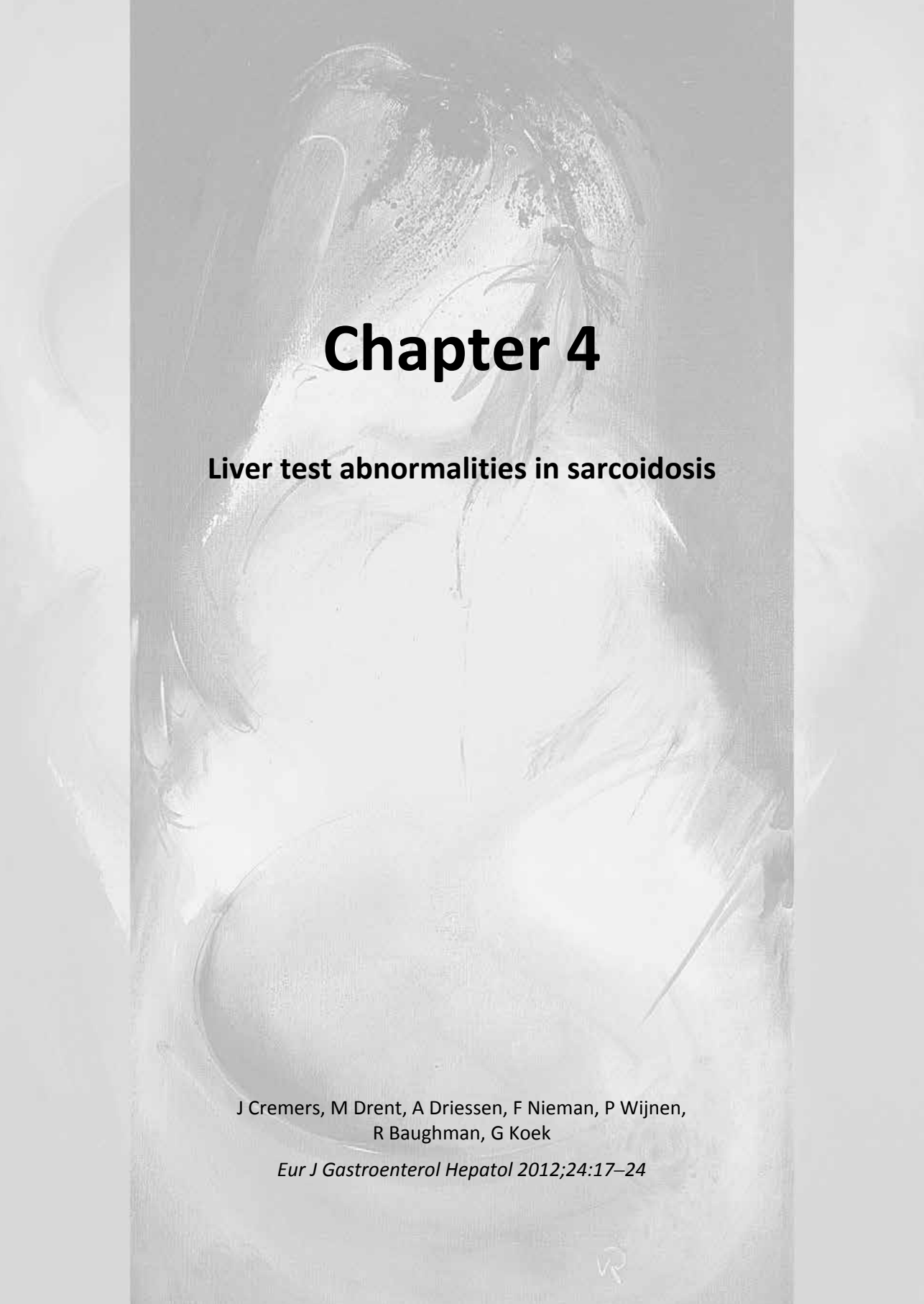

Chapter 4

Liver test abnormalities in sarcoidosis

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Abstract

Background
Sarcoidosis is a multisystemic inflammatory granulomatous disease. The prevalence of hepatic involvement is not clear. The aim of this study was to establish the presence and severity of liver test abnormalities in sarcoidosis.

Methods
Retrospectively, patients with confirmed sarcoidosis (n=837) presenting with the liver test abnormalities (alkaline phosphatase, γ-glutamyl transferase, alanine aminotransferase or aspartate aminotransferase >1.5 times the upper limit of normal (ULN)) who were classified according to severity into mild (zero liver tests ≥3 times the ULN), moderate (one or two liver tests ≥3 times the ULN) and severe (three or four liver tests ≥3 times the ULN) were evaluated. Moreover, the relationship between severity of liver tests and histology was examined.

Results
Liver test abnormalities were found in 204 of 837 patients with chronic sarcoidosis (24.4%), among which 127 (15.2%) were suspected of having hepatic sarcoidosis (79 of 127 males, 111 Caucasian, eight African-American). In 22 of 127 patients (17.3%), a liver biopsy was obtained; 21 were compatible with hepatic sarcoidosis. In these 21 patients, severity of liver test abnormalities was significantly associated with extensiveness of granulomatous inflammation (ρ=0.58, p=0.006) and degree of fibrosis (ρ=0.64, p=0.002). These results remained in the multiple regression analysis when controlled for treatment status, sex, genetics, ethnicity and age.

Conclusion
Liver test abnormalities were present in 24% of the studied patients; in 15% highly because of hepatic involvement of sarcoidosis. Moderate and severe liver test abnormalities seemed to be associated with more advanced histopathological disease. Therefore, in the management of sarcoidosis, for patients with moderate or severe liver test abnormalities a liver biopsy is recommended.

Introduction

Sarcoidosis is a multisystemic inflammatory granulomatous disease of unknown etiology.[1] Typical for sarcoidosis is the presence of non-necrotizing granulomas, affecting lung and lymph nodes in more than 90% of the cases, but it can involve almost every organ system.[2-5] The prevalence of sarcoidosis is estimated at 1 to 40 cases per 100 000, with an age-adjusted annual incidence in the USA of 35.5 per 100 000 for African-Americans and 10.9 per 100 000 for Caucasians.[1]

Extrapulmonary sites, including the liver, are involved in 50% of cases.[4,6,7] Hepatic sarcoidosis mostly affects young individuals between 20 and 40 years old, and is twice as common in African-Americans as in Caucasians.[6-12] Hepatic sarcoidosis is usually asymptomatic.[4,8,13] Only 5–30% of the patients present with atypical clinical signs and symptoms such as nausea, vomiting, abdominal pain in de right upper quadrant, a painful liver during palpation and/or hepatomegaly.[3,8,10-12,14-21] In a minority of patients, hepatic sarcoidosis can be a serious and rapid progressive disease with the occurrence of complications as cirrhosis, portal hypertension, chronic cholestasis and Budd-Chiari syndrome.[3,4,8,10,13,14,17,21-24] Because symptomatic hepatic sarcoidosis is uncommon, studies to investigate the natural history and the diagnostic approach are scarce.

Diagnosis of hepatic sarcoidosis is established when clinicoradiological findings are supported by a compatible histological picture of a biopsy obtained from another organ and/or the liver, after the exclusion of other causes of hepatic granulomas.[1] Guidelines with recommendations for the assessment of hepatic involvement in patients with sarcoidosis presenting with liver test abnormalities are not yet available. Moreover, diagnosis is considered to be a clinical challenge, because of the wide spectrum of disease presentation and course with the absence of specific symptoms, signs and radiological findings.[3,8,10-12,14-21,25] Furthermore, liver test abnormalities can be found in hepatic sarcoidosis; however, the clinical relevance of the severity of these abnormalities is uncertain.[10-12,16,18,19,26] Therefore, the aim of this study was to establish the presence and severity of the liver test abnormalities in a Dutch sarcoidosis population and additionally, to evaluate the relationship between severity of liver test abnormalities and histopathological abnormalities in hepatic sarcoidosis.

Methods

Study design and selection of patients

In this retrospective study, we analysed the data of patients with the established diagnosis sarcoidosis (n=837) who, between 2000 and 2009, visited the ild care centre (interstitial lung diseases including sarcoidosis management and research) of the Maastricht University Medical Centre+ (MUMC+), a tertiary referral centre for the Netherlands.

Of the studied sarcoidosis patients, in 570 (68.1%) a biopsy was obtained: 269 of the lung; 112 of the skin; 145 of a lymph node; 44 of other organs of which 22 of the liver. In patients with typical features of the Löfgren's syndrome and characteristic features of the bronchoalveolar lavage fluid analysis results, no biopsy was obtained. This policy is consistent with the World Association of Sarcoidosis and Other Granulomatous Diseases guidelines.[1,27]

Liver involvement was suspected if one of the following liver tests exceeded the level of the upper limit of normal (ULN) 1.5 times: alanine aminotransferase more than 67.5 U/l for men and more than 52.5 U/l for women, aspartate aminotransferase more than 52.5 U/l for men and more than 45 U/l for women, alkaline phosphatase (ALP) more than 210 U/l for both men and women, γ-glutamyl transferase more than 75 U/l for men and more than 60 U/l for women. All available histological liver biopsies from the histopathologic department of the MUMC+ were examined to cross-check and supplement the list of patients suspected of hepatic sarcoidosis.

Patients with liver test abnormalities that were attributable to drug use (n=12) or other (liver) diseases (n=10) were excluded.

Collection of clinical data

For each patient suspected of hepatic sarcoidosis, we reviewed Mirador, the electronic database of MUMC+, in detail to gather the following items: general information, data concerning the diagnostic process (laboratory abnormalities, radiological findings, liver biopsies) and treatment status. Because of the absence of a standard grading system, we classified the liver test abnormalities according to severity into mild (zero liver tests ≥3 times the ULN), moderate (one or two liver tests ≥3 times the ULN) and severe (three or four liver tests ≥3 times the ULN). Ultrasonographic findings were defined positive (i.e. suggestive for hepatic sarcoidosis) in the presence of hyperechogenic or hypoechogenic spots.[13] Treatment status was established for the distinction between patients treated and patients not treated during the liver biopsy. All data were recorded within 6 months from the time the liver biopsy was performed. For missing follow-up data we contacted the medical practitioners of the secondary care centre where the patient had been treated originally.

Histopathologic examination

Histological examination was performed on formalin-fixed paraffin-embedded material. A panel of histochemical stains was used: haematoxylin and eosin (H/E), Sirius red, reticulin, Periodic Acid-Schiff diastase reaction and Ziehl Nielsen. The aim of these stains was to evaluate the architecture of the liver, the presence and degree of inflammation, steatosis and fibrosis and the distribution of the granulomas. In the granulomas we looked for necrosis, Schaumann's bodies and asteroid bodies. An immunohistochemical analysis was restricted to a cytokeratin 7-stain in order to evaluate the presence of bile ducts, and their possible loss (ductopenia). Polarization of

the liver biopsy was conducted to detect birefringent material. According to a classification modified from Kahi et al.,[17] the extent of granulomatous inflammation was graded. Based on the percentage of liver sample occupied by granulomas the liver biopsies were classified into three groups: 0–25, 26–50, more than 50%. The degree of inflammation was subtyped into mild (no or minimal periportal inflammation), moderate (periportal and minimal lobular inflammation) and severe (periportal and lobular inflammation).[28] Fibrosis was classified according to the classification proprosed by Desmet et al.[28]: grade 1 (periportal fibrous expansion without septa formation), grade 2 (porto-portal septa with intact architecture), grade 3 (portocentral septa with architectural distortion) and grade 4 (cirrhosis). The level of steatosis was determined according to the classification proposed by Kleiner et al.[29]: stage 0, less than 5%; stage 1, more than 5–33%; stage 2, more than 33–66% and stage 3, more than 66% of parenchyma involved by steatosis.

Statistical analysis

For univariate normally distributed variates means and SDs were used. For non-normally distributed variates score minima and maxima were given. For univariate nominal or ordinal variables frequencies and percentages were used. To examine and test associations between ordinal diagnostic scales (liver test abnormalities and histopathology), log-likelihood chi-squares (X_L^2) and Spearman's rank-correlation coefficient (Spearman's ρ) were used. Despite the low statistical power, an attempt was made to use multiple regression analysis to adjust this relationship for other confounding variables (treatment status, age, sex, genetics and ethnicity). Forward selection was used to find a final regression model containing only statistically significant effects. A p value less than 0.05 was considered to represent a statistically significant relationship. SPSS-pc version 16.0 (SPSS Inc., Chicago, Illinois, USA) was used for analysis.

Results

Selection process

A total of 837 patients with a confirmed diagnosis of sarcoidosis visited the ild care centre of the MUMC+ (Figure 4.1). Liver test abnormalities were present in 204 patients (24.4%). Among these, 77 patients were excluded: 55 because of minimal liver test abnormalities (<1.5 times ULN), 12 because of suspected medication induced liver test abnormalities (among others methotrexate, azathioprine and antibiotics), two because of development of colorectal carcinoma with hepatic metastases during sarcoidosis disease course, five because of other liver diseases (viral hepatitis, alcohol induced liver damage and cholelithiasis) and three patients because of liver test abnormalities as a

consequence of pulmonary hypertension due to pulmonary sarcoidosis with resultant cardiac congestion. The remaining 127 patients were included with the suspected diagnosis of hepatic sarcoidosis. In 22 out of 127 patients with liver test abnormalities (17.3%) a liver biopsy was performed. Twenty-one out of 22 biopsies showed granulomas compatible with hepatic sarcoidosis. These 21 biopsies were used for the comparison between severity of liver test abnormalities and histological findings. One liver biopsy was small and fragmented and showed no granulomas, possibly because of a sampling error.

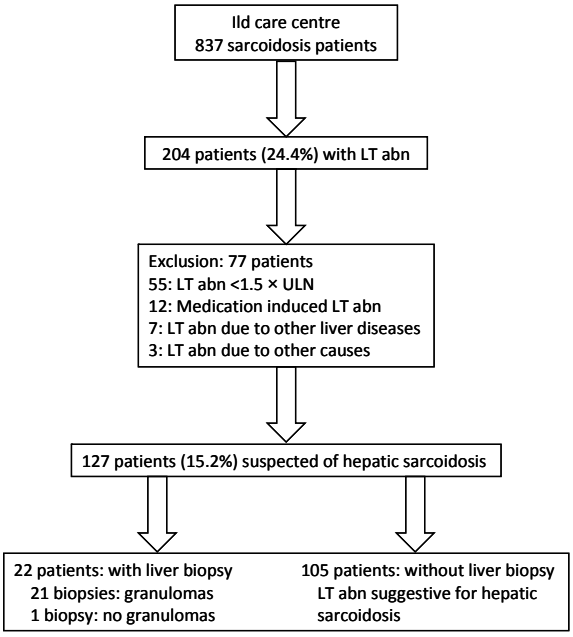

Figure 4.1 Flow diagram inclusion and exclusion of patients. LT abn, liver test abnormalities; ULN, upper limit of normal.

Baseline characteristics

Finally, 127 patients (79 men, 48 women) were suspected of hepatic sarcoidosis. Baseline characteristics are summarized in Table 4.1. The prevalence of suspected symptomatic liver involvement in patients with the confirmed diagnosis sarcoidosis in this group of patients was estimated at 1:7 (15.2%). Mean age of patients with suspected symptomatic hepatic sarcoidosis was 50.5 years (±11.39). Familial involvement was reported in 7.9% of patients and African-American descent in 6.3%.

Liver test abnormalities were in 43.3% cases of cholestatic origin, in 22.8% parenchymatous and in 33.9% combined.

Table 4.1 Baseline characteristics of patients suspected of hepatic sarcoidosis (n=127).

Baseline characteristics	
Age (years)[a]	50.5 (±11.39, 28–83)
Sex[b]	
Male	79/127 (62.2%)
Female	48/127 (37.8%)
Race[b]	
Caucasian	111/126 (88.1%)
African-American	8/126 (6.3%)
Other	7/126 (5.6%)
Average disease duration (years)[a]	7.93 (±5.45, 1–29)
Familial involvement[b]	10/126 (7.9%)
Smoking[b]	15/124 (12.1%)
Liver test abnormalities[b,c]	
Mild	36/127 (28.3%)
Moderate	85/127 (67.0%)
Severe	6/127 (4.7%)
Liver test abnormalities[b]	
Cholestatic	55/127 (43.3%)
Parenchymatous	29/127 (22.8%)
Combined	43/127 (33.9%)

[a] Data are presented as means ±SD with range. [b] Data are presented as absolute numbers with percentages. [c] Liver test abnormalities on the basis of severity: mild, 1.5 x ULN < LTs ≥3 x ULN; moderate, 1 or 2 LTs ≥3 x ULN; severe, 3 or 4 LTs ≥3 x ULN. LT abn, liver test abnormalities; ULN, upper limit of normal.

Histopathological findings

In 17.3% (22 of 127 patients) a liver biopsy was obtained, of which 21 were compatible with hepatic sarcoidosis. Baseline characteristics and the results of histopathological assessment for this subpopulation are summarized in Table 4.2. The percentage of biopsy area occupied by granulomas was estimated to be less than 50% in 71% of the liver biopsies, with granulomas located predominantly mixed portal and parenchymatous (Figure 4.2a and 4.2b). Inflammation was present in all cases, often periportal, but involving the liver parenchyma to a variable degree in 12 patients (57%). In one patient asteroid bodies were found in giant cells situated in granulomas, as illustrated in Figure 4.2c. Ductopenia was present in 33% of cases. Birefringent material was made visible during polarization in a total of six granulomas (Figure 4.2d). Fibrosis grade 3 was noted in five biopsies (24%).

Table 4.2 Baseline characteristics and histopathological findings (n=21).

Baseline characteristics		
Mean age (years)[a]		48(±13, 28–73)
Sex[b]	Male	12 (57%)
	Female	9 (43%)
Race[b]	Caucasian	19 (90%)
	African-American	2 (10%)
Liver test abnormalities[b,c]	Mild	5 (24%)
	Moderate	12 (57%)
	Severe	4 (19%)
Histological features		
Area of liver samples occupied by granulomas[b]	0–25%	10 (48%)
	26–50%	5 (24%)
	>50%	6 (29%)
Non-necrotizing granulomas[b]		21(100%)
Location granulomas[b]	Portal	4 (19%)
	Parenchymatous	4 (19%)
	Mixed	13 (62%)
Asteroid bodies[b]		1 (5%)
Schaumann's bodies[b]		0 (0%)
Ductopenia[b]		7 (33%)
Sinusoidal dilatation[b]		5 (24%)
Birefringent material on polarization[d]		6 granulomas
Cholestatic changes[b]		4 (19%)
Degree of inflammation[b,e]	Mild	9 (43%)
	Moderate	10 (48%)
	Severe	2 (10%)
Degree of fibrosis[b,f]	Stage 0	5 (24%)
	Stage 1	9 (43%)
	Stage 2	2 (10%)
	Stage 3	5 (24%)
	Stage 4	0 (0%)
Degree of steatosis[b]	Stage 0: <5%	12 (57%)
	Stage 1: 5–33%	6 (29%)
	Stage 2: 34–66%	2 (10%)
	Stage 3: >66%	1 (5%)

[a] Data are presented as means ±SD with range. [b] Data are presented as absolute numbers with percentages. [c] Liver test abnormalities on the basis of severity: mild, 1.5 x ULN < LTs ≥3 x ULN; moderate, 1 or 2 LTs ≥3 x ULN; severe, 3 or 4 LTs ≥3 x ULN. [d] Using a polarizing microscope revealed birefringent material, that is, the presence of foreign body material. [e] Degree of inflammation: mild, no or minimal periportal inflammation; moderate, periportal and minimal lobular inflammation; severe, periportal and lobular inflammation.[28] [f] Degree of fibrosis: stage 0, no fibrosis; stage 1, periportal fibrous expansion without septa formation; grade 2, porto-portal septa with intact architecture; grade 3, portocentral septa with architectured distortion; grade 4, cirrhosis.[28] LT, liver test; ULN, upper limit of normal.

For the 21 available liver biopsies, we examined the association between the three main outcome histopathological features and the severity of liver test abnormalities. Severity of liver test abnormalities was significantly and positively associated with the percentage of biopsy area occupied by granulomas (Spearman's ρ=0.58, p=0.006) and degree of fibrosis (Spearman's ρ=0.64, p=0.002). After control for the possible

confounding variables treatment status, sex, genetics, ethnicity and age by multiple regression analysis, severity of liver tests was still significantly associated with extensiveness of granulomatous inflammation (moderate vs. mild beta=0.53, p=0.02; severe vs. mild beta=0.66, p=0.005) and degree of fibrosis (beta=0.22, p=0.24 and beta=0.87, p<0.001, Table 4.3). Sex appeared to be significantly associated with extent of granulomatous inflammation as well (beta=0.39, p=0.04): in females more advanced stages of granulomatous inflammation can be observed. After statistical correction for possible confounders, severity of liver test abnormalities appeared not to be associated with degree of inflammation, but with age and race.

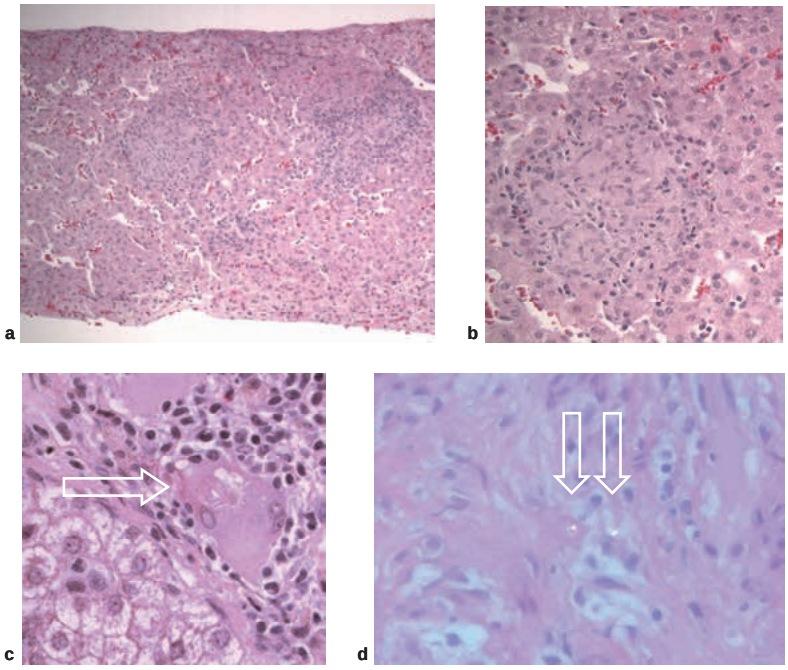

Figure 4.2 Liver biopsy sections from patients diagnosed with hepatic sarcoidosis. **a**. Granulomas located in portal fields and liver parenchyma (H/E, magnification 10x). **b**. Non-necrotizing granuloma consisting of epithelioid histiocytes and giant cells surrounded by a rim of lymphocytes (H/E, magnification 20x). **c**. Asteroid body in a giant cell (H/E, magnification 40x). **d.** Birefringent material during polarization, pointing towards foreign exogenous material as a possible cause for granuloma formation (H/E, magnification 63x). H/E, haematoxylin and eosin.

When the liver biopsy was performed, four patients were pharmacologically treated: two patients with prednisone because of extrahepatic sarcoidosis activity and two patients because of suspected hepatic sarcoidosis (one patient used prednisone and one ursodeoxycholic acid). In multiple regression analysis, treatment status did not appear to disturb the association between liver tests and histology.

Table 4.3 Results of the three final regression analysis models (n=21).

	b	SE	β	p value	95% CI of b	
					Lower	Upper
1. Area of liver sample occupied by granulomas; variance explained = 0.50						
Constant	0.73	0.32	-	0.04	0.05	1.41
Severity LT abn[c]						
Moderate vs. mild	0.91	0.36	0.53	0.02*	0.15	1.66
Severe vs. mild	1.43	0.45	0.66	0.005*	0.48	2.38
Sex						
Female vs. male	0.67	0.30	0.39	0.04*	0.04	1.29
2. Degree of inflammation; variance explained = 0.62[a]						
Constant	0.03	0.36	-	0.95	–0.73	0.78
Age	0.03	0.01	0.66	<0.001*	0.02	0.05
Ethnicity						
Afr-Am vs. Cauc	0.85	0.32	0.39	0.02*	0.18	1.53
3. Degree of fibrosis, variance explained = 0.59[b]						
Constant	0.60	0.34	-	0.09	–0.10	1.30
Severity LT abn[c]						
Moderate vs. mild	0.48	0.40	0.22	0.24	–0.36	1.32
Severe vs. mild	2.40	0.50	0.87	<0.001*	1.34	3.46

* Statistically significant at p<0.05. [a] Degree of inflammation: mild, no or minimal periportal inflammation; moderate, periportal and minimal lobular inflammation; severe, periportal and lobular inflammation.[28] [b] Degree of fibrosis: stage 0, no fibrosis; stage 1, periportal fibrous expansion without septa formation; grade 2, porto-portal septa with intact architecture; grade 3, portocentral septa with architectured distortion; grade 4, cirrhosis.[28] [c] Liver test abnormalities on the basis of severity: mild, 1.5 x ULN < LTs ≥3 x ULN; moderate, 1 or 2 LTs ≥3 x ULN; severe, 3 or 4 LTs ≥3 x ULN. b, unstandardized regression coefficient; CI, confidence interval; LT abn, liver test abnormalities; SE, standard error for b; ULN, upper limit of normal; β, standardized regression coefficient.

Radiological findings

Liver ultrasound evaluation in 13 patients with histological confirmed hepatic sarcoidosis, revealed positive results in six cases (sensitivity 46.2%).

Discussion

To the best of our knowledge, this was the first study demonstrating that severity of liver test abnormalities was significantly related with the degree of fibrosis and extensiveness of the granulomatous inflammation in sarcoidosis. Diagnosis of hepatic sarcoidosis is difficult, as diagnostic criteria are lacking and guidelines with recommendations for diagnosis and follow-up of patients suspected of hepatic sarcoidosis are not available. However, because hepatic sarcoidosis can be a serious and rapid progressive disease, a correct diagnosis without delay is important.

There has been conflicting evidence to both support and refute the rationale that liver test abnormalities are associated with the extent of the severity of liver involvement. In patients with asymptomatic hepatic sarcoidosis, Lebacq and Heller[26]

found ALP to be more reliable than ɣ-glutamyl transferase in predicting sarcoid liver involvement. Baughman et al.[30] found higher levels of various liver tests, especially ALP, in patients with liver sarcoidosis. In patients with abnormal liver tests, hepatic granulomas were more often seen compared to patients with normal liver tests.[19] In contrast, others did not find this relationship.[11] However, liver parameters were not classified according to severity and the study group was small in the latter study (n=15).[12] In 20 patients with advanced inflammatory changes, higher levels of ALP, transaminases and fibrosis around hepatic granulomas in the liver were reported. However, this study did not grade liver tests and remained descriptive.[10]

Every physician managing patients with sarcoidosis should be alert about the possibility of the development of hepatobiliary disorders. On the bases of the results of the present study, we made a first attempt for a diagnostic algorithm in the diagnosis and follow-up of patients with sarcoidosis presenting with liver test abnormalities (Figure 4.3). In patients with liver test abnormalities above 1.5 times the ULN for more than 3 months, a careful follow-up is recommended. Unfortunately, studies with arguments for follow-up of liver tests in patients with different degrees of liver test abnormalities are lacking.

For the classification of severity of liver test abnormalities in our study we found no distinction between cholestatic and parenchymatous liver tests.

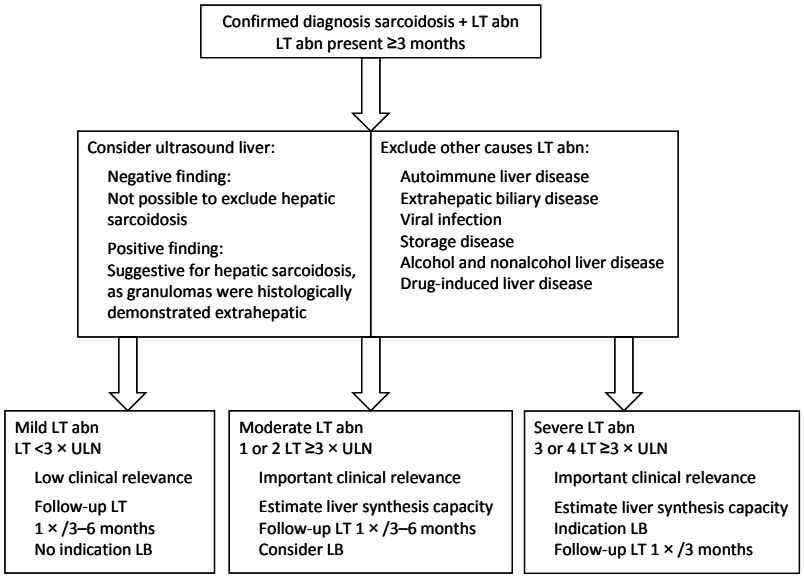

Figure 4.3 Diagnostic algorithm sarcoidosis patients with liver test abnormalities. LB, liver biopsy; LT, liver tests; LT abn, liver test abnormalities.

Although liver biopsy might be useful in diagnosing hepatic sarcoidosis, there are some major disadvantages. First of all, percutaneous liver biopsy is an invasive procedure with the risk of major complications (among others haemorrhage, perforation of gall-bladder or colon, pneumothorax and biliary peritonitis) estimated to occur in 0.09%–3.1% of cases.[31-33] Moreover, liver biopsy might be accompanied with a lot of anxiety and postbiopsy discomfort.[31] Furthermore, as a biopsy specimen represents as few as 1 in 50.000 of the total mass of the liver,[34] sampling error may be a possible consequence. However, Hercules and Bethlem[11] demonstrated that false-negative results in liver biopsy were unlikely.

Whether radiological examination might be an alternative for detection of hepatic sarcoidosis is not clear. In the present study, a sensitivity of 46.2% was found for liver ultrasound. This implicates that it is not useful in the exclusion of hepatic sarcoidosis in the case of an absence of hyperechogenic or hypoechogenic spots. Positive findings of liver ultrasound were suggestive for hepatic sarcoidosis, especially when signs of extrahepatic granulomas consistent with sarcoidosis were found. In line with the previous studies in which a sensitivity of 53-59.2% for conventional liver ultrasound in the detection of general hepatic lesions was shown, it appeared of limited value in the confirmation of hepatic sarcoidosis.[35,36]

In sarcoidosis patients with severe liver test abnormalities during a minimum of 3 months, advanced stages of fibrosis must be suspected. In addition, in patients with moderate liver test abnormalities during a minimum of 3 months, advanced stages of fibrosis cannot be excluded. A liver biopsy to assess the disease severity to guide a more or less aggressive pharmacological approach is recommended. The occurrence of hepatic complications because of advanced stages of fibrosis (stage 3 and 4) in our study was estimated at almost 25%, which is in accordance with the finding of bridging fibrosis and cirrhosis in 8-26% of cases in previous studies.[4,14,17] If liver test abnormalities progress to advanced stages, it is recommended to adjust the diagnostic approach according to Figure 4.3.

One of the major limitations of this study is the small number of liver biopsies. Prospective studies are warranted to establish liver sarcoidosis in a respectable number of patients.

Furthermore, although the studied patient population was gathered in a tertiary referral centre, the severity mainly refers to the chronicity of the sarcoidosis patients. Hence this study appears to be representative for chronic Dutch sarcoidosis patients. However, conclusions must be interpreted with caution.

In conclusion, in the studied Dutch chronic sarcoidosis population liver test abnormalities were present in 24% of patients; in 15% highly likely because of hepatic involvement of sarcoidosis. Severity of liver test abnormalities appeared to be related to the degree of fibrosis and extensiveness of granulomatous inflammation in hepatic sarcoidosis. In the management of sarcoidosis patients presenting with severe liver test abnormalities, a liver biopsy is strongly recommended, whereas, in patients with moderate liver test abnormalities a liver biopsy should be considered.

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