

CLINICAL STUDIES

Hepatic granulomas: histological and molecular pathological approach to differential diagnosis—a study of 442 cases

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Abstract

Background/Aims: The incidence of hepatic granulomas is reported in 2–15% of liver biopsies. This study was carried out to evaluate the incidence and aetiology of hepatic granulomas in a German Institute of Pathology with specialization in liver diseases. **Methods:** A retrospective case review was performed on 12 161 liver biopsies of the Institute of Pathology (University of Cologne) between 1996 and 2004. Aetiology was determined according to histomorphological changes, clinicopathological data and liver tissue polymerase chain reaction (PCR) for detection of diverse putative pathogens in the liver tissue. **Results:** Four hundred and forty-two liver biopsies revealed granulomatous lesions (3.63%). Two hundred and fifteen cases (1.77% of all biopsies and 48.64% of granulomatous lesions) were diagnosed as primary biliary cirrhosis. In 37 cases (0.3% of all biopsies and 8.37% of granulomatous lesions), the diagnosis of sarcoidosis was established. A positive PCR result for an infectious pathogen was obtained in 15 samples (3.39%) [*Bartonella henselae* ($n = 2$), *Listeria* ($n = 3$), *Mycobacterium tuberculosis* ($n = 3$), *Yersinia pseudotuberculosis* ($n = 1$), cytomegalovirus ($n = 2$), Epstein–Barr virus ($n = 4$)]. In six cases, a putative diagnosis was established according to the report of clinical conditions. In 11 cases (2.48%), drugs were the putative causative agent. In 158 cases (36%) a definite diagnosis could not be established. **Conclusions:** Hepatic granulomas have a broad range of underlying aetiologies. With a combined histological, clinical, serological, and molecular approach, we were able to clarify the cause in 64% of the cases. Owing to the diverse prognosis and therapeutic implications, a detailed interdisciplinary workup of all liver biopsies with granulomatous lesions is mandatory.

Hepatic granulomas are a frequent finding in unselected liver biopsies and may be associated with a broad spectrum of infectious and non-infectious disorders. Granulomas are focal accumulations of modified macrophages and may display a surrounding rim of lymphocytes and fibroblasts (1). The incidence of hepatic granulomas ranges from 2 to 15% (2–5) compared with 2–3% in bone marrow biopsies (6). Granulomas may occur in the liver in a wide variety of disorders, some of which are primary to the liver, but most are part of a generalized disease process. The spectrum of aetiologies varies with the geographical conditions as well as the characteristics of the patient population (3–5). Reported causes of hepatic granulomas are immunological diseases, like primary biliary cirrhosis (PBC), hypersensitivity, foreign materials,

neoplasms and miscellaneous conditions (7). Also, infectious agents are common, such as *Mycobacteria* ssp., *Yersinia* ssp., *Toxoplasma gondii* (*T. gondii*) and *Bartonella henselae* (*B. henselae*) (8).

The diagnosis of granulomas in the liver is usually made by the histopathologist and requires careful histological evaluation of the granulomas themselves and of any associated changes in the surrounding liver tissue (hepatitis, cholangitis, cholestasis, non-specific inflammation) (9). Distinctive microscopic features of hepatic granulomas like the size, shape and distribution of the granulomas, the presence and type of necrosis, presence, number and size of giant cells may narrow the differential diagnosis to several possibilities. Granulomas with caseating necrosis can be found in infectious diseases like tuberculosis (TBC),

non-caseating necrosis in cat-scratch disease and other infectious conditions. Granulomas with fibrosis are typical for sarcoidosis. Granuloma formation without fibrosis can be drug induced, and granulomas with bile duct destruction are the typical lesion of PBC. However, the diagnosis often remains uncertain on the basis of histology alone. Denk *et al.* (9) provided a diagnostic frame with four categories [(i) see the cause, (ii) know the cause, (iii) suspect the diagnosis, (iv) cause unknown] that is still the state of the art in classifying the reliability of disease diagnosis in liver granulomas.

Thus, establishing the aetiology necessitates a broad-based clinical and pathological approach (1). In the case of an infectious aetiology, similar to other organs, special stains and molecular analyses, such as polymerase chain reaction (PCR) and hybridization approaches, can contribute towards identification of the culpable antigen (10, 11).

This investigation was performed in order to re-evaluate the prevalence and aetiology of hepatic granulomas in liver biopsies using an integrative approach of histopathology, clinical findings and molecular analyses. Our findings demonstrate that a definite cause can be obtained in the majority of cases and necessitate a detailed interdisciplinary workup of liver biopsies with granulomas.

Material and methods

Liver tissue samples

A retrospective case review was performed on liver biopsies collected from the files of the Institute of Pathology of the University Hospital Cologne, Germany, between 1996 and 2004 to identify all liver biopsies with hepatic granulomas. The pathology reports were reviewed, and liver biopsies with the diagnosis of granuloma formation were collected. Biopsies revealing lipogranulomas (suggesting origin from steatohepatitis) or mineral oil granulomas (lipid droplets surrounded by macrophages and lymphocytes) were excluded from this study.

Histopathological analysis

Liver tissue specimens were processed according to standard histological techniques and stained for microscopic analysis with haematoxylin and eosin (H&E), Elastica van Gieson (EVG), Gomori and Period acid Schiff Reagent after diastase (D-PAS). Ziehl–Neelsen stain (ZN) was performed in 20 cases and Whartin–Starry stain in five cases. Histopathology was evaluated independently by two experienced liver pathologists

(U. D., H. U. K.). Granulomas were defined as focal aggregates of macrophages and epithelioid and multinucleated giant cells surrounded by lymphocytes. Hepatic and cholangitic changes were distinguished. The presence of necrosis and caseation, epithelioid cells and giant cells of Langhans type was noted. Granuloma-associated or independent fibrosis and inflammation were recorded.

Clinical data

The clinical data available were reviewed ($n = 370$). The data included a medical history, autoantibodies, immunoglobulins, hepatitis B and C serology, drug history and further specialized tests. The results were analysed in order to determine the aetiology of hepatic granulomas.

Polymerase chain reaction

Three micrometre paraffin sections were deparaffinized in xylene by incubation at 65 °C for 20 min. The deparaffinized sections were washed in 500 µl of 100% ethanol. After lysis in 300 µl of proteinase K buffer (500 µg/ml proteinase K) (Invitrogen, Karlsruhe, Germany), 50 mM Tris-HCL, pH 7.4, 5 mM ethylene diamine tetra-acetic acid, pH 8, and 1% sodium dodecyl sulphate, nucleic acids were extracted using the DNA extraction kit from Purgene (Minneapolis, MN, USA) according to the instructions of the manufacturer. Finally, the DNA was resolved in 25 µl H₂O. PCR was performed using a Biometra-Trio Thermal Cycler (Biometra, Göttingen, Germany) in a final volume of 50 µl [5 µl of extracted DNA, 5 µl reaction buffer (Sigma, Germany), 5 µl dNTPs (1 mM), 32.8 µl H₂O, 2 µl of each primer (10 µM) and 0.2 µl of TAQ polymerase (Sigma)]. Nested PCRs were performed in order to detect and amplify the following viral, bacterial or protozoal DNA: *B. henselae*, *Listeria monocytogenes*, *Mycobacterium tuberculosis* (*M. tuberculosis*) and *Mycobacterium avium* (*M. avium*), *Yersinia enterocolitica* (*Y. enterocolitica*) and *Yersinia pseudotuberculosis* (*Y. pseudotuberculosis*), cytomegalovirus (CMV), Epstein–Barr virus (EBV) and *T. gondii*. Primers recognizing the human haemochromatosis (HFE) gene locus were used in a reference PCR as a positive control for DNA quality (F1: ATGGATGCC AAGGAGTTC-GAACC, F2: GCCATAATTACCTCCTCAGGCAC, R1: TTCTCAGCT CCTGGCTCTCATC, R2: TCGAACC-TAAAGACGTATTGCC). Primers for *B. henselae*, *M. tuberculosis* and *M. avium*, *Y. enterocolitica* and *Y. pseudotuberculosis*, CMV, EBV and *T. gondii* were described recently (11–13). For *Listeria*, the following primers were used: F1/F2: CCCGCCTGGTCTAACTGGATTG, R1:

GGATGGAAATTTGGTCGG CGTACA and R2: GTA-TATCGTGACTTACGAGG.

After initial denaturation for 5 min at 94 °C, the following annealing temperatures and numbers of cycles were used for nested PCR amplification: *B. henselae*: PCR I, II: 60 °C, 40 cycles, *L. monocytogenes*: PCR I, II: 62 °C, 35 cycles, *M. tuberculosis*: PCR I: 66 °C, 35 cycles, PCR II: 70 °C, 32 cycles, *M. avium*: PCR I, II: 60 °C, 40 cycles, *Y. enterocolica* and *Y. pseudotuberculosis*: PCR I, II: 58 °C, 35 cycles, CMV: PCR I: 58 °C, 35 cycles, PCR II: 54 °C, 30 cycles, EBV: PCR I: 58 °C, 35 cycles, PCR II: 57 °C, 40 cycles, *T. gondii*: 62 °C, 35 cycles and HFE: PCR I: 60 °C, 30 cycles, PCR II: 58 °C, 30 cycles.

The PCR products were subsequently resolved by electrophoresis in a 3% agarose gel and visualized by ethidium bromide staining.

Results

Incidence of hepatic granulomas

Over the 8-year period of this study, 12 161 liver biopsies were referred to the Institute of Pathology. These consisted largely of needle biopsies although small numbers of wedge resections were carried out. Four hundred and forty-two (3.63%) samples revealed granulomatous lesions (423 needle biopsies, 19 wedge resections). These lesions consisted of single granulomas and granulomatous hepatitis or cholangitis.

Hepatic granulomas with typical or suspicious histopathological lesions

Primary biliary cirrhosis

Two hundred and fifteen cases (1.77% of all biopsies and 48.64% of granulomatous lesions) were diagnosed as PBC in a two-step procedure. Firstly, PBC was diagnosed in case of a definite histological picture consisting of periductal granulomas with bile duct destruction and lympho-plasmacytic portal infiltration ($n = 174$). Secondly, cholangitis was found and portal granulomas were seen without demonstrable bile duct destruction ($n = 41$). For all cases of PBC, clinical and laboratory data showed either an elevation of antimitochondrial antibodies (AMAs) or anti-nuclear antibodies (in case of AMA-negative PBC) or alkaline phosphatase (AP) elevation.

Sarcoidosis

The diagnosis of sarcoidosis was established in 37 cases (0.30% of all biopsies and 8.37% of granulomatous

lesions) when histology was compatible (non-caseating, epithelioid cell granulomas without topographical association, lack of demonstrable mycobacteria, often tendency of fibrosis) ($n = 30$). In seven cases, histology was not typical (granulomas without fibrosis, necrosis). In all cases, clinical/serological data were at least highly suggestive for sarcoidosis (typical clinical features like hilar adenopathy, raised angiotensin-converting enzyme, typical radiological findings).

Q-fever

Fibrin-ring granulomas were seen in one case, suggesting the diagnosis of Q-fever, which was confirmed by clinical and serological data.

Further investigations (after exclusion of primary biliary cirrhosis, sarcoidosis and Q-fever)

The remaining biopsies ($n = 189$) were further investigated by detailed histopathological analyses, evaluation of clinical data and molecular pathological approach.

Demographical and clinical data of patients with hepatic granulomas (after exclusion of primary biliary cirrhosis, sarcoidosis and Q-fever)

The evaluation of the clinical and laboratory data of the remaining patients ($n = 189$) is summarized. Ninety-two biopsies were from men and 97 were from women (mean age 52 years, range 5–78). Aspartate transaminase ($n = 102$) ranged from 20 to 518 U/L; alanine aminotransferase ($n = 98$) ranged from 18 to 582. The following conditions, which might explain granuloma formation, were reported: a positive serology of Lues II ($n = 2$), a positive serology of acute cat-scratch disease ($n = 1$) and Hodgkin's disease ($n = 2$). In addition, the following conditions, which do not explain granuloma formation, were reported: hepatitis B serostatus ($n = 87$) revealing chronic hepatitis B in two cases, hepatitis C serostatus ($n = 86$) revealing chronic hepatitis C in two cases, positive HIV serostatus ($n = 1$), colitis ulcerosa ($n = 1$) and acute myeloid leukaemia ($n = 1$). Furthermore, fever was reported in five cases and drugs in 35 cases.

Results of polymerase chain reaction amplification of infectious agents

DNA could be extracted and analysed in 184 of 189 cases indicated by a reference PCR for the human HFE gene locus. In all cases, DNA could be amplified, and

negative controls carried along each case, remained negative, assuring a contamination-free DNA extraction procedure. In 15 liver tissue specimens (3.39% of hepatic granulomas), an infectious agent was detected and the liver histology was compatible with the analysis result. These cases represent cases with a formerly unknown aetiology of granuloma formation. *B. henselae* was detected in two liver biopsies. Disseminated epithelioid cell granulomas were seen and at least some granulomas revealed a necrotizing reaction in all cases. *L. monocytogenes* was seen in three cases. Histology revealed a granulomatous hepatitis with multiple ill-defined granulomas without necrosis. *M. tuberculosis* was found in three cases. Epithelioid cell granulomas were found to be portal and extra-portal. In every case, caseating necrosis was seen, but mycobacteria could not be identified with ZN stain even retrospectively. *Y. pseudotuberculosis* was detected in one liver biopsy showing multiple non-caseating granulomas. CMV DNA was demonstrated by PCR in two liver biopsies. Ill-defined histiocytic granulomas were seen within the parenchyma, together with microabscesses and with cholangitic changes as well as endothelitis. In four samples, EBV was detected. In these cases, small, ill-defined histiocytic granulomas were seen within hepatic parenchyma, together with a sinusoidal lymphocytic infiltrate, cholangitis and endothelitis of the central veins. Different histopathological forms of hepatic granulomas are presented exemplarily in Figure 1.

Anamnestic data about medication

Detailed clinical data about drug history were available in 35 cases. The following medications, known to cause granuloma formation in the liver, were reported (14), suggesting the suspected diagnosis of drug-induced granuloma formation ($n = 11$, 2.48%): glibencalmide ($n = 2$), oral contraceptives ($n = 4$), hydrochlorothiazide ($n = 2$), aspirin ($n = 1$) and interferon ($n = 2$).

Diagnostic algorithm

With these data, we were able to interpret the aetiological conditions according to Denk's approach for the diagnosis and interpretation of hepatic granulomas. The diagnostic algorithm is shown in Figure 2. The aetiology of hepatic granulomas could be established in 15 cases, which had been submitted without clinical and serological data by interpretation of PCR results and histomorphological changes.

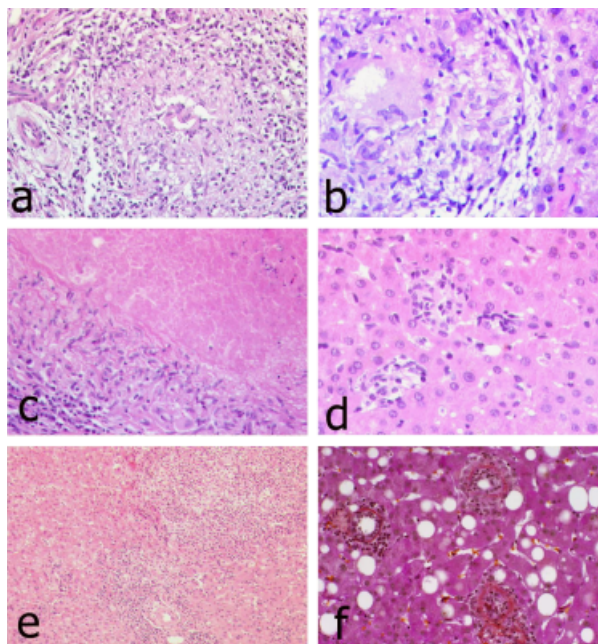


Fig. 1. (a) Florid lesion in primary biliary cirrhosis with granuloma formation around a destroyed bile duct. [H&E, $\times 250$]. (b) Granuloma of sarcoidosis with epithelioid cells and giant cell formation. No necrosis is obvious (H&E, $\times 400$). (c) Caseating necrosis in a granuloma with epithelioid cells. *Mycobacterium tuberculosis* was found by polymerase chain reaction (PCR) in liver tissue (H&E, $\times 400$). (d) Small, ill-defined intralobular histiocytic granulomas in a liver biopsy positive for EBV in PCR (H&E, $\times 250$). (e) Liver biopsy with ill-defined periportal granulomas with granulation tissue from a patient with systemic cat scratch disease. *Bartonella henselae* was detected by PCR of liver tissue (H&E, $\times 250$). (f) Fibrin ring granulomas with intralobular localization. In the centre of small granulomas, a fibrin ring surrounds a vacuole in a patient with serologically proven Q-fever (Pearse, $\times 250$).

Discussion

The current retrospective study presents a comprehensive analysis of a large series of liver biopsies with granulomas. The referred liver biopsies mirror a representative spectrum of liver diseases of a significantly higher proportion of adult patients. The evaluation was performed by histomorphology, clinicopathological data and molecular pathological analyses and the results were evaluated according to Denk's criteria.

In our study, we did not 'see the cause' of granuloma formation (group 1 of Denk) (e.g. ova of *Schistosoma*, mycobacteria with ZN stain, foreign bodies) in any biopsy in contrast to Satti *et al.*, (5) who could show that the main causes of hepatic granulomas in Saudi Arabia were schistosomiasis and TBC, schistosomiasis

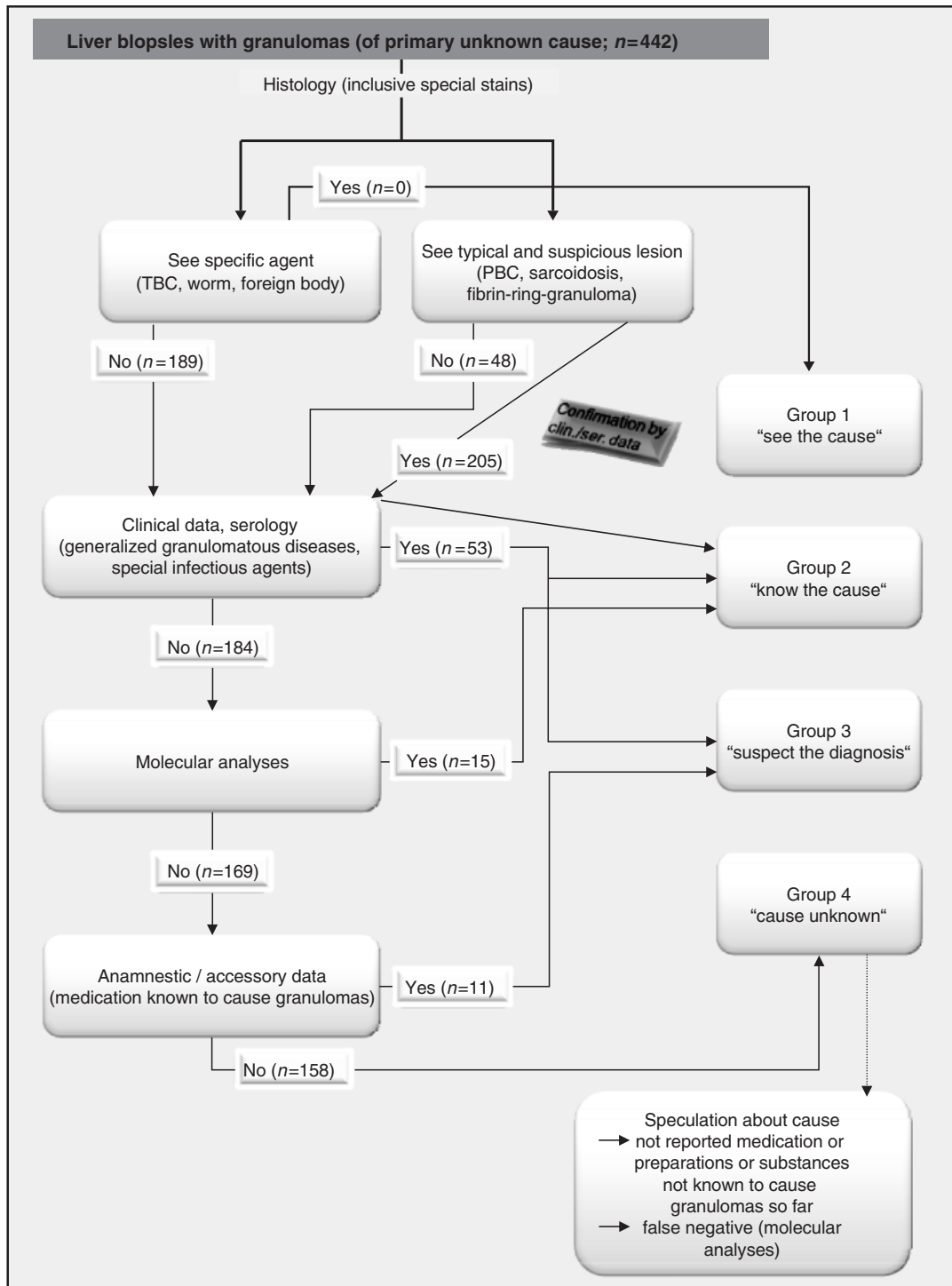


Fig. 2. Diagnostic algorithm according to Denk’s approach for the diagnosis and interpretation of hepatic granulomas.

is rarely seen in European countries. In a Turkish study analysing hepatic granulomas, TBC was found as to be an aetiological agent in 20% of cases (8). In the present study, TBC accounted for three cases. However, the diagnosis was suggested by the presence of caseating

necrosis and could be confirmed by the positive PCR result for *M. tuberculosis*. ZN stain did not identify the bacteria; thus, the *M. tuberculosis*-positive cases were categorized after PCR as group 2 (‘know the cause’). For this group, the knowledge of clinical and

serological data or the result of molecular analyses is inevitable. Biopsies that are categorized in this group either have specific histopathological changes which like the florid bile duct lesion in PBC, or granulomatous changes, which can be non-characteristic. Serological and clinical data like AMA and AP elevation in PBC or like the clinical diagnosis Hodgkin's disease are necessary to establish the cause of granuloma formation in the liver.

We performed PCR for the analysis of infectious agents. We were able to find infectious agents in 15 cases, thus knowing the cause of granuloma formation.

The incidence and aetiology of infectious diseases depend mainly on the geographical location. In addition to TBC, we were able to detect *B. henselae* by PCR in the liver in two cases, reflecting a systemic manifestation of cat-scratch disease, which is rarely described (15, 16). We found EBV, CMV and *Listeria* as well as *Y. pseudotuberculosis* in a small number of cases, which are known to induce granulomatous liver lesions (12). We could demonstrate that infectious diseases are an important aetiological factor for granuloma formation in the liver and that a molecular pathological approach is an efficient tool for establishing the diagnosis.

Even in the absence of a clear diagnosis the histopathologist is able to suggest the most fruitful line of investigation when 'suspecting the diagnosis', the third category of the Denk scheme (9). In this context, drug-induced granuloma formation plays an important role. Drugs, toxins and environmental factors like occupational exposure to metals like beryllium may lead to granuloma formation. More than 60 drugs (like allopurinol, aspirin and oral contraceptives) have been incriminated as the cause of hepatic granulomas (14). The knowledge of the medication is inevitable and the clinician should provide these data for the pathologist.

In 36% of the cases, a diagnosis could not be established according to the morphological changes or available clinical data and were defined as Denk group 4 'cause unknown'. In a proportion of these cases, incomplete clinical data and medication might play a role and a more intense search for infectious agents and the precise knowledge of clinical conditions might elucidate the diagnosis. Other cases might belong to the group of idiopathic granulomatous hepatitis, a condition of granulomatous hepatitis without known aetiology, which often occurs in young adults (17–19).

There have been several major studies investigating the incidence of hepatic granulomas from different countries over the past decades (2, 3, 5, 20).

As reported by other authors, who found hepatic granulomas in up to 2–15% of patients, our relatively

low incidence in 3.63% of liver biopsies most likely reflects the geographical and socioeconomical situation of a highly industrialized European country. Furthermore, with advances in immunological and virological testing and therapeutic options the spectrum of causative diseases and indication for biopsy has been changing. Hughes, Wagoner, Klatskin and Cunningham, who analysed hepatic granulomas in western countries, described sarcoidosis and TBC as the most common aetiologies (21–24). While PBC was underestimated until the early 1980s (4, 21–27), it is now the leading cause of hepatic granulomas in western countries (2, 3, 7, 20). After the discovery of hepatitis C virus, a small percentage of granulomas were interpreted as an epiphenomenon of chronic hepatitis C (2, 28–30). In addition, some recent studies have been selective, because patients have been referred to a specialized centre with a suspected clinical diagnosis or because of investigation only among children (10, 27).

In our study, consistent with investigations from other western countries, PBC was the leading cause of granuloma formation in the liver. PBC accounted for 48.64% of liver biopsies containing granulomas. McCluggage and Sloan (3) reported the diagnosis of PBC in 55% of granulomatous liver lesions in Northern Ireland, Gaya *et al.* (2) found PBC in 23.8% in a single centre of the UK and Dourakis *et al.* (20) reported PBC in 62% of liver biopsies with hepatic granulomas in a large series from Greece. Differences in the proportion of PBC in these studies may be explained as a consequence of epidemiological factors, selection bias and differences in the indication for liver biopsy in cases of suspected PBC (31).

The second leading cause of granuloma formation in the liver was sarcoidosis. Sarcoidosis accounted for 8.37% of liver biopsies, containing granulomas. Similar results were reported by Gaya *et al.* (2), McCluggage and Sloan (3) and Dourakis *et al.* (20). Consistent with other authors, we found that infectious diseases are the next most common aetiology of hepatic granulomas, followed by drugs and toxins (7, 14).

In conclusion, we have identified granulomas in 3.63% of liver biopsies. The clinical profile responsible for the presence of granulomas in the liver is wide and requires an extensive examination. A careful histopathological examination is required for the establishment of the diagnosis. PBC and sarcoidosis are the most frequent aetiologies for granuloma formation in the liver. PCR on the liver tissue is potentially useful in analysing infectious aetiological conditions of granuloma formation. Drugs and toxins should be included in the differential diagnosis.

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