SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2023; 40 (3); e2023031 DOI: 10.36141/svdld.v40i3.14221 

# Diagnostic value of elevated serum angiotensin-converting enzyme and lymphopenia in patients with granulomatous hepatitis

*Thomas El Jammal[1], François Dhelft[1], Pierre Pradat[2], François Bailly[3], Fabien Zoulim[3], Arnaud Hot[4], Maxime Fauter[1], Ayoub Drissi-Bakhkhat[1], Isabelle Durieu[5], Jean-Christophe Lega[5], Yvan Jamilloux[1], Pascal Sève[1,6]*

[1]Department of Internal Medicine, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Université Claude Bernard Lyon; [2]Centre de Recherche Clinique, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Université Claude Bernard Lyon; [3]Department of Hepatology, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, Université Claude Bernard Lyon; [4]Department of Internal Medicine, Hôpital Edouard Herriot, Hospices Civils de Lyon, Université Claude Bernard Lyon; [5]Department of Internal Medicine, Hôpital Lyon Sud, Hospices Civils de Lyon, Université Claude Bernard Lyon; [6]Hospices Civils de Lyon, Pôle IMER, Lyon F-69003, France; Univ. Lyon, University Claude Bernard Lyon

**Abstract.** *Background and aim*: Granulomatous hepatitis (GH) is associated with various aetiologies, especially inflammatory and infectious disorders. Sarcoidosis is a granulomatous disease in which the liver is the fourth most affected organ. Since epithelioid cell granulomas are not specific to sarcoidosis and since most patients with hepatic sarcoidosis are asymptomatic, valuable diagnostic biomarkers are needed to support the diagnosis of sarcoidosis. This study proposes to assess the diagnostic value of serum angiotensin converting enzyme (sACE) and lymphopenia in GH for sarcoidosis. *Method*: We retrospectively analyzed the records of 90 patients referred to the internal medicine or hepatogastroenterology departments of the Lyon University Hospital (Lyon, France) between March 2002 and January 2020 in a context of GH. *Results*: In our tertiary center, 38 patients with sarcoidosis were identified among 73 patients with GH. Lymphopenia had a high specificity (88.9%), which increased when combined with elevated sACE (90.9%). Interestingly, specificity increased in patients under 50 years old (100%). *Conclusions*: Those results suggests that lymphopenia and sACE may be valuable biomarkers for sarcoidosis diagnosis in GH when combined, especially in younger patients.

**Key words:** granulomatous hepatitis, sarcoidosis, angiotensin converting enzyme, lymphopenia

## Introduction

Granulomatous hepatitis (GH) is a common finding in unselected liver biopsy specimens and is associated with a broad spectrum of infectious and non-infectious disorders. Based on clinical studies, the prevalence of granulomatous liver disease ranges from 2.4% to 15% of all liver biopsy specimens (1). The etiologic spectrum mostly depends on the geographic area and on the characteristics of the source population (2). In Western countries, the two main causes of GH are sarcoidosis and primary biliary cirrhosis (PBC) (3–5).

Sarcoidosis is a systemic inflammatory disease of unknown origin characterised by the presence of non-caseating granulomas various organs, the most frequently affected ones after the lung and intrathoracic lymph nodes being the skin, eyes and extrathoracic lymph nodes, with liver involvement usually being the fourth or fifth most common extra-thoracic manifestation (2,6,7). The incidence of hepatic sarcoidosis is underestimated in clinical practice as most

Received: 1 February 2023
Accepted: 30 July 2023
Correspondence: Prof. Pascal Seve, Service de Médecine Interne
Hôpital de la Croix-Rousse
103 grande rue de la Croix-Rousse, F-69004, Lyon, France
E-mail: pascal.seve@chu-lyon.fr

patients are asymptomatic. In antemortem studies, liver involvement in sarcoidosis is estimated to range from 5 to 30% of the patients and up to 70% in an autopsy study (8–13). Thus, asymptomatic sarcoidosis might be largely underestimated. Nevertheless, establishing an accurate diagnosis of sarcoidosis in those patients is important since it orientates therapeutic management.

Serum angiotensin-converting enzyme (sACE) is one of the most commonly used biomarkers in sarcoidosis. However, its sensitivity varies from 60% to 80% and mainly depends on the organs involved (14–18). Lymphopenia is also a classic feature of sarcoidosis. It occurs in more than half of patients with sarcoidosis and is associated with a chronic disease course (19).

In patients with GH, the diagnosis of sarcoidosis is usually established by the combination of chest imaging and laboratory tests and can be confirmed by pathological analysis of an extra-hepatic tissue sample (20). Since liver biopsy is an invasive procedure and given the relatively high prevalence of granulomas on liver biopsies (from 2.4% to up to 15% of all liver biopsy specimens), the search for diagnostic biomarkers for hepatic sarcoidosis is a key issue (1).

We therefore undertook this retrospective study to investigate the value of elevated sACE and lymphopenia, alone or in combination, as diagnostic biomarkers of hepatic sarcoidosis in patients with GH. A clear assessment of those tests' metrics can provide insights for the diagnostic management of suspected liver sarcoidosis, especially to avoid unnecessary investigations.

## Methods

### *Patients*

We retrospectively analyzed the records of 90 consecutive adult patients referred for the etiological workup of GH to the internal medicine or hepato-gastroenterology departments of the Lyon University Hospital (Lyon, France) between March 2002 and January 2020. The study excluded cases of GH in patients with an already-diagnosed disease.

### *Diagnosis and definition*

Granulomatous hepatitis was defined as the presence of granulomas on the histological examination of a liver biopsy. We then analyzed the pathology reports, which were generated by different pathologists. Patients underwent a standard screening protocol that included a chest X-ray or CT scan, standard blood tests (including a complete blood cell count, CBC), a C-reactive protein assay, serologic tests for autoimmune hepatitis and hepatitis virus, measurement of sACE and a QuantiFERON®-TB Gold Plus test or a tuberculin skin test. Diagnostic screening for sarcoidosis included lymph node or skin biopsies if there was any clinical evidence of skin involvement. Some patients underwent minor salivary gland biopsy (MSGB), transbronchial lung biopsy, bronchoalveolar lavage fluid analysis, or 18F-FDG Positron Emission Tomography (PET). Other investigations were guided by the clinical context.

Serum ACE was considered elevated if its level exceeded the upper limit indicated by our laboratory (52 IU/L) by one standard deviation (SD=16 IU/L). Patients taking an ACE inhibitor, systemic corticosteroids, or immunosuppressive/immunomodulatory therapies were excluded from the analysis.

Lymphopenia was considered significant if the lymphocyte count was below our laboratory reference value ($<1x10^9$/L). Because children often have higher lymphocyte counts than adults, patients younger than 18 years were excluded from the study.

Patients were diagnosed with sarcoidosis according to the guidelines of the American Thoracic Society/World Association of Sarcoidosis and Other Granulomatous diseases (ATS1/WASOG) (21,22).

### *Data collection*

Patients' demographic data, clinical presentation, biological and imaging findings at disease onset and during follow-up were collected.

### *Statistical analysis*

Data were analyzed with R software version 4.1.2. (R Foundation for Statistical Computing, Vienna, Austria). Variables were excluded from the final analysis if there were more than 20% of missing values. Categorical variables were reported as n (%) and continuous variables as mean (±standard deviation, SD) in the case of a normal distribution, or median and interquartile range (IQR) in the case of a skewed distribution. For categorical variables, comparisons between groups were performed using the Chi-squared

test or Fisher's exact test, as appropriate. Continuous variables were compared using the Wilcoxon-Mann-Whitney test in the case of a skewed distribution and with Student's t-test in the case of a normal distribution. Sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) were calculated for lymphopenia and sACE, alone and in combination. Receiver operating characteristic (ROC) curves were plotted; areas under the curve (AUC) with 95% confidence intervals (95% CI) were reported. Optimal cut points for lymphocyte count, sACE and age were computed with the *Optimal.cutpoints* package. A multivariable analysis was conducted by creating nine different generalized linear models (one for each combination of those three variables) in order to better determine what variable was contributing the most to the positive diagnosis. All the tests were two-tailed and a p-value<0.05 was considered significant.

*Ethics*

The Lyon Granulomatous Hepatitis Study received approval from the local ethics committee in July, 2020 and was registered on www.clinicaltrials.gov (NCT04459897).

## Results

*Overall population characteristics*

Ninety adult patients with GH underwent pre-specified screening for sarcoidosis. After the exclusion of 12 subjects whose CBC and sACE measurements were missing, plus five patients treated with ACE inhibitors or systemic corticosteroids, 73 patients were finally analyzed (Figure 1).

The characteristics of the study population are shown in Table 1.

The mean age of disease onset was 47.2 (±14.4) years. Forty-two patients (58%) were female and 45 (61.6%) were of Caucasian descent. Thirty (41%) patients were clinically asymptomatic. The most frequent symptom was altered general status (34%), associated with fever in 13% of the cases. Two patients had abdominal pain and two others had clinical evidence of portal hypertension. Three other patients were diagnosed through a systematic biopsy of the liver during a cholecystectomy procedure. Forty-four (60.3%) patients had cholestasis (γ-glutamyl transferase greater than three times the upper limit of normal and/or alkaline phosphatase greater than once and a half the upper limit of normal) (23). Cholestasis was associated with an elevation of aminotransferases (> *twice the upper limit of normal)* in 18 patients (24.7%). Within the sarcoidosis population, apart from liver involvement, the lymph nodes (81.6%), the spleen, and the lung (both 34.2%) were the most commonly affected organs.

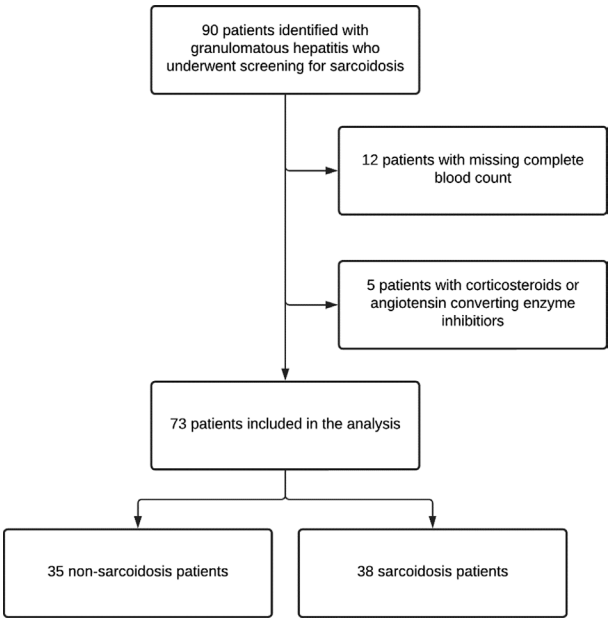


**Figure 1.** Flowchart depicting the selection process of patients in the current study.

A chest CT scan or a PET scan was performed in 11 patients classified as having idiopathic GH and did not show any abnormalities suggestive of an extrahepatic disease. Table 2 depicts the distribution of aetiologies across the population.

*Comparison between sarcoidosis and non-sarcoidosis patients*

The characteristics of the 38 patients with sarcoidosis are reported in Table 1. The mean age in this group was 47.2 (14.4) years, 52.6% were female and 60.5% were Caucasians. Seventeen (45.9%) sarcoidosis patients were clinically asymptomatic, while 11 (29.7%) had an altered general status, among whom three had fever (8.1%). One patient in the sarcoidosis group had portal hypertension (without cirrhosis). The demographic and clinical characteristics

**Table 1.** Summary table of patients with sarcoidosis compared with patients with other diagnoses.

| | **Non-sarcoidosis N=35** | **Sarcoidosis N=38** | **p.value** |
|---|---|---|---|
| Age | 49.3 (14.4) | 47.2 (14.4) | 0.536 |
| Sex: | | | 0.518 |
| Female | 22 (62.9%) | 20 (52.6%) | |
| Male | 13 (37.1%) | 18 (47.4%) | |
| Ethnicity: | | | 0.901 |
| Africa | 3 (8.57%) | 3 (7.89%) | |
| Asia | 1 (2.86%) | 0 (0.00%) | |
| Europe | 22 (62.9%) | 23 (60.5%) | |
| North Africa | 9 (25.7%) | 12 (31.6%) | |
| Lymphopenia: | 5 (14.3%) | 13 (34.2%) | 0.089 |
| Lymphocytes ($10^9$/L) | 1.60 [1.20;2.20] | 1.17 [0.71;1.46] | 0.004 |
| Increased sACE: | 11 (36.7%) | 21 (58.3%) | 0.132 |
| sACE (UI/L) | 46.0 [27.8;79.5] | 70.0 [52.0;104] | 0.013 |
| Clinical signs: | | | 0.908 |
| AGS alone | 7 (19.4%) | 8 (21.6%) | |
| AGS + fever | 5 (13.9%) | 3 (8.11%) | |
| Abdominal pain | 1 (2.78%) | 1 (2.70%) | |
| PH | 1 (2.78%) | 1 (2.70%) | |
| pruritus | 0 (0.00%) | 1 (2.70%) | |
| asymptomatic | 13 (36.1%) | 17 (45.9%) | |
| other | 9 (25.0%) | 6 (16.2%) | |
| Sarcoidosis organ involvement | | | |
| Liver | - | 38 (100%) | |
| Lymph nodes | - | 31 (81.6%) | |
| Spleen | - | 13 (34.2%) | |
| Lung | - | 13 (34.2%) | |
| Joints | - | 8 (21.1%) | |
| ENT | - | 3 (7.9%) | |
| Eye | - | 3 (7.9%) | |
| Skin | - | 2 (5.3%) | |
| Bone | - | 1 (2.6%) | |
| Neurological | - | 1 (2.6%) | |
| Digestive tract | - | 1 (2.6%) | |
| Kidney | - | 1 (2.6%) | |
| Bone marrow | - | 1 (2.6%) | |
| Heart | - | 0 (0%) | |

Abbreviations: AGS: altered general status; ENT: ear, nose, throat; PH: portal hypertension; sACE: serum angiotensin converting enzyme.

**Table 2.** Summary table of aetiologies of granulomatous hepatitis patients in the series.

| Etiology | N (%) (total = 73) |
|---|---|
| IHG | 12 (16.4%) |
| Infections | |
| Tuberculosis | 5 (6.8%) |
| Coxiella burnetii | 1 (1.4%) |
| Rickettsiosis | 1 (1.4%) |
| HBV* | 1 (1.4%) |
| HCV* | 1 (1.4%) |
| Immunological/inflammatory | |
| Sarcoidosis | 38 (52.0%) |
| Autoimmune hepatitis | 2 (2.7%) |
| Behçet disease | 1 (1.4%) |
| Sclerosing cholangitis | 1 (1.4%) |
| Primary biliary cirrhosis | 1 (1.4%) |
| Miscellaneous | |
| Steatohepatitis | 2 (2.7%) |
| Cirrhosis | 2 (2.7%) |
| Hepatocellular carcinoma | 1 (1.4%) |
| Rendu Osler Weber disease | 1 (1.4%) |
| Lymphocytic lymphoma | 1 (1.4%) |
| Toxic | 1 (1.4%) |
| Immune reconstitution after HSCT | 1 (1.4%) |

Abbreviations: HBV/HCV: hepatitis B/C virus; HSCT: hematopoietic stem cell transplant; IGH: idiopathic granulomatous hepatitis; * no serological testing before biopsy.

did not differ from the comparison with non-sarcoid GH patients. However, cholestasis was significantly more frequent in the group of patients with sarcoidosis than in the non-sarcoid GH group (68.4% versus 42.9%, p=0.03). Moreover, an elevation of aminotransferases was present in eight (21.1%) patients of the sarcoidosis group versus 15 (43.0%) in the other group (p=0.05).

*Serum angiotensin converting enzyme level and lymphocyte count*

The median sACE level was 60.0 IU/L (IQR, 38.0-90.0). In patients with sarcoidosis, the median sACE was 70.0 IU/L (IQR, 52.0-104.0) versus 46.0 IU/L (IQR, 27.8-79.5) in patients without sarcoidosis (p=0.013). sACE was elevated in 21 subjects with sarcoidosis (58.3%) versus 11 (36.7%) subjects with an alternate diagnosis (p=0.132). In the false positive group, the final diagnoses included: tuberculosis, PBC, rickettsiosis and idiopathic GH.

In subjects with sarcoidosis, the median lymphocyte count was 1.17 $x10^9$/L (IQR, 0.71-1.46), whereas, in subjects without sarcoidosis, the median lymphocyte count was 1.60 x $10^9$/L (IQR, 1.20-2.20) (p=0.004). Overall, lymphopenia was observed in 18 subjects. It was observed in 13 (34.2%) subjects with sarcoidosis and in five (14.3%) patients with an alternate diagnosis (p=0.089*)*. In patients with an alternate diagnosis, the final diagnoses included: tuberculosis, idiopathic GH and viral B hepatitis.

*Reliability of sACE and lymphopenia for the diagnosis of sarcoidosis*

To better determine which variable was contributing the most to the diagnosis of sarcoidosis between age, lymphopenia, and elevated sACE, a multivariable analysis in which nine generalized linear models were established was done. It appears that the model that took into account the lymphocyte count and the age was the best one for sarcoidosis prediction (AUC = 0.771, Figure S1). This analysis also revealed that sACE was associated with the less performant models for predicting sarcoidosis (Table S1).

By applying the pre-specified cut-off of 68 IU/L, the Se of sACE elevation for the diagnosis of sarcoidosis in patients with GH was 46.9%, Sp was 66.7%, PPV was 62.5% and NPV was 51.4% (Table 3).

The AUC was 0.664 (95% CI, 0.524–0.816) (Figure 2). Based on the ROC curve analysis, the optimal sACE cut-off to maximize both specificity and sensitivity was found at 60 IU/L. This threshold resulted in a Se of 59.4% and a Sp of 63.0% for the diagnosis of sarcoidosis in patients with GH.

With the threshold of 1.0x$10^9$/L, lymphopenia had a Se of 37.5% for the diagnosis of sarcoidosis in patients with GH, while Sp was 88.9%, PPV was 80.0% and NPV was 54.5%. The AUC was 0.766 (95% CI, 0.642–0.891) (Figure 3). Based on this ROC curve, the optimal cut-off for lymphopenia to maximize both specificity and sensitivity was 1.4x$10^9$/L, resulting in a Se of 71.9%, a Sp of 70.4%, with a PPV of 74.2% and a NPV of 67.9% for the diagnosis of sarcoidosis in patients with GH.

**Table 3.** Diagnostic performances of lymphopenia and sACE either isolated or in combination for sarcoidosis diagnosis in granulomatous hepatitis patients.

| | | Se | Sp | PPV | NPV | PLR | NLR |
|---|---|---|---|---|---|---|---|
| **All** | Elevated sACE | 0.469 | 0.667 | 0.625 | 0.514 | 1.406 | 0.797 |
| | Lymphopenia | 0.375 | 0.889 | 0.800 | 0.545 | 3.375 | 0.703 |
| | Lymphopenia + Elevated sACE | 0.421 | 0.909 | 0.889 | 0.476 | 4.632 | 0.637 |
| **>=50 years** | Elevated sACE | 0.400 | 0.714 | 0.600 | 0.526 | 1.400 | 0.840 |
| | Lymphopenia | 0.333 | 0.786 | 0.625 | 0.524 | 1.556 | 0.848 |
| | Lymphopenia + Elevated sACE | 0.222 | 0.833 | 0.667 | 0.417 | 1.333 | 0.933 |
| **<50 years** | Elevated sACE | 0.529 | 0.615 | 0.643 | 0.500 | 1.376 | 0.765 |
| | Lymphopenia | 0.412 | 1.000 | 1.000 | 0.565 | Inf | 0.588 |
| | Lymphopenia + Elevated sACE | 0.600 | 1.000 | 1.000 | 0.556 | Inf | 0.400 |
| **With optimal cutpoint** | Elevated sACE | 0.594 | 0.630 | 0.655 | 0.567 | 1.603 | 0.645 |
| | Lymphopenia | 0.719 | 0.704 | 0.742 | 0.679 | 2.426 | 0.399 |
| | Lymphopenia + Elevated sACE | 0.556 | 0.615 | 0.750 | 0.400 | 1.444 | 0.722 |

Abbreviations: sACE: serum angiotensin converting enzyme, NPV: negative predictive value, PPV: positive predictive value. Values were computed on 59 patients to take into account missing values.

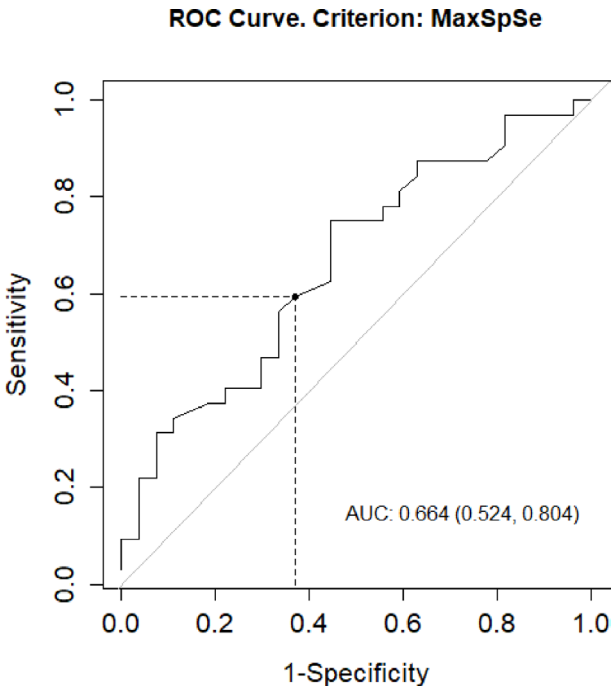


**Figure 2.** Receiver operating characteristics curve for serum angiotensin converting enzyme (optimal cutpoint according to the maximization for both Sensitivity and Specificity is represented with a dotted line). Abbreviations: AUC: area under curve, ROC: receiver operating curve.

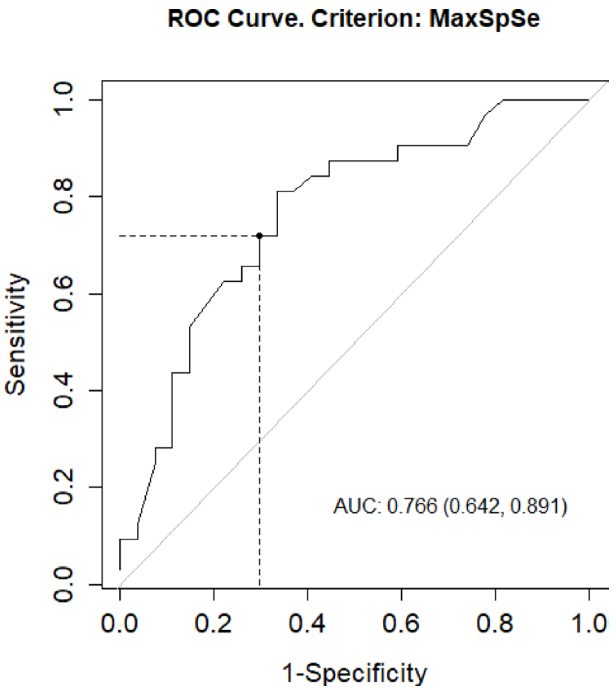


**Figure 3.** Receiver operating characteristics curve for lymphopenia (optimal cutpoint according to the maximization for both Sensitivity and Specificity is represented with a dotted line) Abbreviations: AUC: area under curve, ROC: receiver operating curve.

The combination of an elevated sACE and lymphopenia (using standard cut-off values) resulted in a Se of 42.1%, Sp of 90.9%, PPV of 88.9% and NPV of 47.6% for the diagnosis of sarcoidosis in such patients. When applying the optimal cut-off values (i.e., $1.4x10^9$/L for lymphopenia and 60 IU/L for sACE), the Se for the combination was 55.6%, Sp was 61.5%, PPV was 75.0% and

NPV was 40.0% for the diagnosis of sarcoidosis in patients with GH.

*Comparison of reliability indices according to patients' age:*

We then wondered whether the reliability of the biomarkers could depend on patient age. patients aged <50 years, Se for the combination of elevated sACE and lymphopenia was 60.0%, Sp was 100%, PPV was 100% and NPV was 55.6%. Lymphopenia and sACE had poorer diagnostic performance in patients over the age of 50, whether alone or in combination. Se and Sp for the two combined markers were 22.2% and 83.3%, respectively. Again, sACE sensitivity was lower when both markers were combined. PPV and NPV were respectively 66.7% and 41.7%. The combination of sACE and lymphopenia increased the positive likelihood ratio (PLR) in the overall population as well as in patient under 50.

## Discussion

Here, lymphopenia had a strong specificity for the diagnosis of sarcoid hepatitis and the combination of an elevated sACE with lymphopenia increases their value as diagnostic biomarkers for sarcoid hepatitis. Patients who already had a known etiologic diagnosis at the time of the liver biopsy were excluded, which could explain a different etiologic distribution compared with the main series reported in Western countries (1). Here, about one sixth of patients were experiencing idiopathic GH and 52% were diagnosed with sarcoidosis. Not having a diagnosis at the time of the liver biopsy clearly modifies the source population and thus the distribution of aetiologies. Indeed, not all liver biopsies are taken into account, but only those performed in patients without previously known disease. This probably explains the greater proportion of sarcoidosis and idiopathic granulomatosis in patients for whom the pre-test probability of having these pathologies is greater in the absence of clinical elements of orientation. The monocentric nature of the study, performed in a tertiary center, may also have played a role in the modification of the distribution of aetiologies. As seen in the studies by Coash and colleagues and Geri and colleagues, sarcoidosis was the most common cause of GH in our cohort (1,4). Among sarcoidosis patients, 68.4% had cholestasis, which is the most frequently reported laboratory abnormality (70-90%) in sarcoidosis hepatitis (1,2,24,25). Only one sarcoidosis patient had portal hypertension. In the literature, portal hypertension has been reported in 3 to 20% of cases of sarcoidosis hepatitis (2,26).

Bunting and colleagues first assessed the diagnostic value of elevated sACE in a cohort of 70 patients with systemic sarcoidosis and reported a Se of 63% and a specificity of 93% (27). Ungprasert and colleagues evaluated the diagnostic value of elevated sACE in a large cohort of 3277 patients with systemic sarcoidosis and reported a Se of 41% and a Sp of 89.9% (28). In this study, sACE had a slightly higher Se (51.4%) along with a lower Sp (67.9%), which is close to the most frequently described values for sACE Se and Sp in sarcoidosis in the literature (29). These differences are probably related to the comparison of different populations across studies. Indeed, in the study by Ungprasert and colleagues, the initial population was larger than that of a tertiary center such as ours, with a higher frequency of moderate forms that are less likely to have high sACE levels. In addition, our study focused on sarcoidosis hepatitis, which is the main limitation when comparing to other studies that included systemic sarcoidosis.

Lymphopenia occurs in 26 to 50% of cases of sarcoidosis (19,30) and has been reported as one of the most frequent hematological abnormalities (31,32). Therefore, lymphopenia is mostly observed in patients with active sarcoidosis and in patients who are more likely to evolve toward a chronic disease (33,34). A few studies evaluated the value of the combination of elevated sACE and lymphopenia as biomarkers for the diagnosis of sarcoidosis. In a recent study of 996 patients with uveitis, Cotte and colleagues showed that the combination of elevated sACE and lymphopenia enhanced the specificity to 99%, the PPV to 74% and the NPV to 89.5% for the diagnosis of sarcoid uveitis (35). The present study showed that the combination of elevated sACE and lymphopenia enhanced the specificity to 90.9%, the PPV to 88.9% and the NPV to 47.6% for diagnosing sarcoidosis in patients with GH. However, in this cohort, around one fifth of patients with sarcoidosis had both a normal sACE and no lymphopenia. Finally, this study also showed that if combining both parameters (elevated sACE and lymphopenia) in patients aged <50 years, Se increases to 60.0%, Sp and PPV reach 100%, with the NPV being 55.6%. Contrarily, diagnostic performance at the pre-defined

threshold (1000/mm3) was lower in patients over 50 years of age.

The high positive predictive value of the combination of both sACE and lymphopenia is probably a valuable biomarker in younger patients with granulomatous hepatitis. In a selected population (especially patients under 50 years old) in which hepatic sarcoidosis is suspected, the combination of a lymphocyte count below 1000/mm3 and an elevated sACE over 68 U/l strongly suggests a sarcoidosis diagnosis. On the contrary, a normal lymphocyte count and a normal sACE dosage are not sufficient to eliminate sarcoidosis.

The present study has several limitations. First, its retrospective and monocentric design includes an indication bias because not all diagnostic investigations were performed in all cases. Moreover, we had to deal with missing data and thus exclude some variables from the final analysis. Also, since our patients were selected from a tertiary center, some already had a long past medical history before being referred to our department and thus might differ from daily-care patients. This can make an important difference since sACE is often elevated while lymphocyte count is usually low during the active phase of the disease while being normal later in patients with inactive sarcoidosis (34). We also excluded patients who underwent corticosteroids or immunosuppressive treatments to avoid inhomogeneity in a small population and because corticosteroids could have an impact on the lymphocyte count. Therefore, the population in this study is probably less severe than a population with non-treatment-naïve patients. This study focused only on sACE and lymphopenia since those biomarkers are easily accessible in daily routine in most centers worldwide. More sensitive and specific serum biomarkers, and in particular the soluble interleukin-2 receptor (sIL-2R) is an alternative that was not studied here (36). Indeed, this dosage is not routinely available in many countries yet despite its promising diagnostic metrics. The purpose of the present study was to primarily focus on sACE and lymphopenia metrics, and therefore no biomarker selection was performed. Finally, our results are to be interpreted in the context of GH with a prevalence of sarcoidosis of more than 50% in our source population. This prevalence is much higher than in other studies comprehensively identifying GH and probably increased the PPV and decreased the actual NPV of sACE and lymphopenia (3,37).

## Conclusions

Serum angiotensin converting enzyme and lymphopenia are simple laboratory tests that appear to be useful alone or in combination in diagnosing patients with sarcoidosis. The combination of both an elevated sACE and lymphopenia strongly suggest a diagnosis of sarcoidosis in granulomatous hepatitis patients. Further studies should focus on the prognostic value of such a combination in other organs involved in sarcoidosis and in larger cohorts.

**Conflict of Interest:** Each author declares that he or she has no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

## References

1. Coash M, Forouhar F, Wu CH, Wu GY. Granulomatous liver diseases: a review. J Formos Med Assoc. 2012 Jan;111(1):3–13.
2. Deutsch-Link S, Fortuna D, Weinberg EM. A Comprehensive Review of Hepatic Sarcoid. Semin Liver Dis. 2018;38(3):284–97.
3. Gaya DR, Thorburn D, Oien KA, Morris AJ, Stanley AJ. Hepatic granulomas: a 10 year single centre experience. J Clin Pathol. 2003 Nov;56(11):850–3.
4. Geri G, Cacoub P. [Hepatic granulomas]. Rev Med Interne. 2011 Sep;32(9):560–6.
5. Wainwright H. Hepatic granulomas. European Journal of Gastroenterology & Hepatology. 2007 Feb;19(2):93–5.
6. Syed U, Alkhawam H, Bakhit M, Companioni RAC, Walfish A. Hepatic sarcoidosis: pathogenesis, clinical context, and treatment options. Scand J Gastroenterol. 2016 Sep;51(9):1025–30.
7. Branson JH, Park JH. Sarcoidosis—hepatic involvement: presentation of a case with fatal liver involvement, including autopsy findings and review of the evidence for sarcoid involvement of the liver as found in the literature. Annals of Internal Medicine. 1954 Jan 1;40(1):111–45.
8. Judson MA. Hepatic, splenic, and gastrointestinal involvement with sarcoidosis. Semin Respir Crit Care Med. 2002 Dec;23(6):529–41.
9. Morimoto T, Azuma A, Abe S, Usuki J, Kudoh S, Sugisaki K, et al. Epidemiology of sarcoidosis in Japan. Eur Respir J. 2008 Feb;31(2):372–9.
10. Judson MA, Boan AD, Lackland DT. The clinical course of sarcoidosis: presentation, diagnosis, and treatment in a large white and black cohort in the United States. Sarcoidosis Vasc Diffuse Lung Dis. 2012 Oct;29(2):119–27.
11. Cozier YC, Berman JS, Palmer JR, Boggs DA, Serlin DM, Rosenberg L. Sarcoidosis in black women in the United States: data from the Black Women's Health Study. Chest. 2011 Jan;139(1):144–50.
12. Kennedy PTF, Zakaria N, Modawi SB, Papadopoulou AM, Murray-Lyon I, du Bois RM, et al. Natural history of hepatic sarcoidosis and its response to treatment. Eur J Gastroenterol Hepatol. 2006 Jul;18(7):721–6.
13. Iwai K, Oka H. SARCOIDOSIS. REPORT OF TEN AUTOPSY CASES IN JAPAN. Am Rev Respir Dis. 1964 Oct;90:612–22.
14. Lieberman J. Elevation of serum angiotensin-converting-enzyme (ACE) level in sarcoidosis. Am J Med. 1975 Sep;59(3):365–72.

15. K A, Jf K. Diagnostic value of serum angiotensin converting enzyme activity in lung diseases. Thorax. 1976 Oct 1;31(5):552–7.
16. Silverstein E, Friedland J, Kitt M, Lyons HA. Increased serum angiotensin converting enzyme activity in sarcoidosis. Isr J Med Sci. 1977 Oct;13(10):995–1000.
17. Studdy P, Bird R, James DG. Serum angiotensin-converting enzyme (SACE) in sarcoidosis and other granulomatous disorders. Lancet. 1978 Dec 23;2(8104–5):1331–4.
18. Nosal A, Schleissner LA, Mishkin FS, Lieberman J. Angiotensin-I-converting enzyme and gallium scan in noninvasive evaluation of sarcoidosis. Ann Intern Med. 1979 Mar;90(3):328–31.
19. Sweiss NJ, Salloum R, Gandhi S, Ghandi S, Alegre ML, Sawaqed R, et al. Significant CD4, CD8, and CD19 lymphopenia in peripheral blood of sarcoidosis patients correlates with severe disease manifestations. PLoS ONE. 2010 Feb 5;5(2):e9088.
20. Grunewald J, Grutters JC, Arkema EV, Saketkoo LA, Moller DR, Müller-Quernheim J. Sarcoidosis. Nat Rev Dis Primers. 2019 04;5(1):45.
21. Hunninghake GW, Costabel U, Ando M, Baughman R, Cordier JF, du Bois R, et al. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. Sarcoidosis Vasc Diffuse Lung Dis. 1999 Sep;16(2):149–73.
22. Crouser ED, Maier LA, Wilson KC, Bonham CA, Morgenthau AS, Patterson KC, et al. Diagnosis and Detection of Sarcoidosis. An Official American Thoracic Society Clinical Practice Guideline. Am J Respir Crit Care Med. 2020 Apr 15;201(8):e26–51.
23. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. Journal of Hepatology. 2009 Aug;51(2):237–67.
24. Flamm SL. Granulomatous liver disease. Clin Liver Dis. 2012 May;16(2):387–96.
25. Moreno-Merlo F, Wanless IR, Shimamatsu K, Sherman M, Greig P, Chiasson D. The role of granulomatous phlebitis and thrombosis in the pathogenesis of cirrhosis and portal hypertension in sarcoidosis. Hepatology. 1997 Sep;26(3):554–60.
26. Bihari C, Rastogi A, Kumar N, Rajesh S, Sarin SK. Hepatic Sarcoidosis: Clinico-pathological characterization of symptomatic cases. Acta Gastroenterol Belg. 2015 Sep;78(3):306–13.
27. Bunting PS, Szalai JP, Katic M. Diagnostic aspects of angiotensin converting enzyme in pulmonary sarcoidosis. Clin Biochem. 1987 Jun;20(3):213–9.
28. Ungprasert P, Carmona EM, Crowson CS, Matteson EL. Diagnostic Utility of Angiotensin-Converting Enzyme in Sarcoidosis: A Population-Based Study. Lung. 2016 Feb;194(1):91–5.
29. Baudin B. L'enzyme de conversion de l'angiotensine I (ECA) dans le diagnostic de la sarcoïdose. Pathologie Biologie. 2005 Apr 1;53(3):183–8.
30. Crouser ED, Lozanski G, Fox CC, Hauswirth DW, Raveendran R, Julian MW. The CD4+ lymphopenic sarcoidosis phenotype is highly responsive to anti-tumor necrosis factor-{alpha} therapy. Chest. 2010 Jun;137(6):1432–5.
31. Gupta D, Rao VM, Aggarwal AN, Garewal G, Jindal SK. Haematological abnormalities in patients of sarcoidosis. Indian J Chest Dis Allied Sci. 2002 Dec;44(4):233–6.
32. C B, C L, B W. [Biological manifestations of sarcoidosis]. Ann Med Interne (Paris). 2001 Feb 1;152(1):34–8.
33. Morell F, Levy G, Orriols R, Ferrer J, De Gracia J, Sampol G. Delayed cutaneous hypersensitivity tests and lymphopenia as activity markers in sarcoidosis. Chest. 2002 Apr;121(4):1239–44.
34. Vagts C, Ascoli C, Fraidenburg DR, Baughman RP, Huang Y, Edafetanure-Ibeh R, et al. Unsupervised Clustering Reveals Sarcoidosis Phenotypes Marked by a Reduction in Lymphocytes Relate to Increased Inflammatory Activity on 18FDG-PET/CT. Frontiers in Medicine [Internet]. 2021 [cited 2022 Jun 11];8. Available from: https://www.frontiersin.org/article/10.3389/fmed.2021.595077
35. Cotte P, Pradat P, Kodjikian L, Jamilloux Y, Seve P. Diagnostic value of lymphopaenia and elevated serum ACE in patients with uveitis. Br J Ophthalmol. 2021 Oct;105(10):1399–404.
36. Eurelings LEM, Miedema JR, Dalm VASH, van Daele PLA, van Hagen PM, van Laar JAM, et al. Sensitivity and specificity of serum soluble interleukin-2 receptor for diagnosing sarcoidosis in a population of patients suspected of sarcoidosis. PLoS One. 2019;14(10):e0223897.
37. Dourakis SP, Saramadou R, Alexopoulou A, Kafiri G, Deutsch M, Koskinas J, et al. Hepatic granulomas: a 6-year experience in a single center in Greece. European Journal of Gastroenterology & Hepatology. 2007 Feb;19(2):101–4.

# Appendix

## Supplementary file

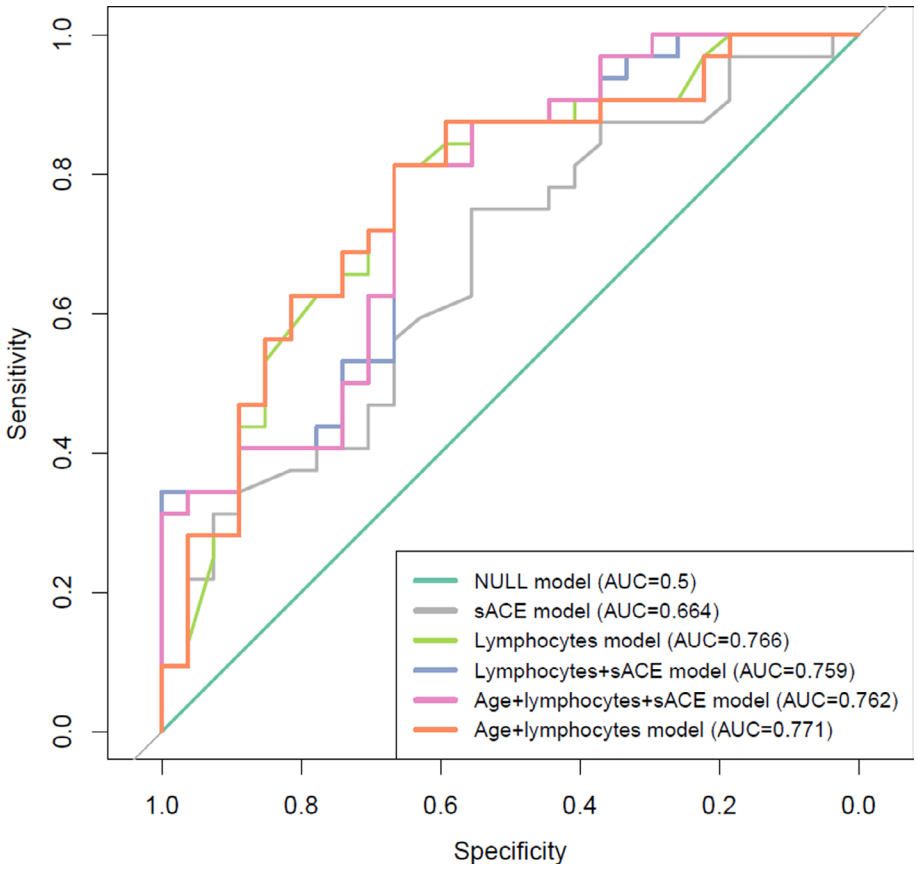


**Figure S1. Receiving operator characteristics curves for the tested multivariable models.** *AUC values are displayed for each model. Abbreviations: sACE: serum angiotensin converting enzyme; AUC: area under curve*

**Table S1.** Table summary of the tested multivariable models.

| | **Sarcoidosis** | | **Sarcoidosis** | | **Sarcoidosis** | | **Sarcoidosis** | | **Sarcoidosis** | | **Sarcoidosis** | | **Sarcoidosis** | | **Sarcoidosis** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Predictors*** | ***Odds Ratios*** | ***CI*** | ***Odds Ratios*** | ***CI*** | ***Odds Ratios*** | ***CI*** | ***Odds Ratios*** | ***CI*** | ***Odds Ratios*** | ***CI*** | ***Odds Ratios*** | ***CI*** | ***Odds Ratios*** | ***CI*** | ***Odds Ratios*** | ***CI*** |
| (Intercept) | 10.82** | 2.71 – 55.80 | 0.43 | 0.14 – 1.17 | 12.38* | 1.26 – 165.64 | 0.32 | 0.03 – 2.89 | 4.51 | 0.68 – 35.68 | 5.92 | 0.25 – 149.26 | 5.09 | 0.12 – 263.16 | 3.89 | 0.22 – 84.60 |
| Lymphocytes | 0.23** | 0.08 – 0.54 | | | 0.23** | 0.08 – 0.54 | | | 0.28** | 0.10 – 0.66 | 0.23 | 0.03 – 1.53 | 0.23 | 0.03 – 1.53 | 0.28** | 0.10 – 0.66 |
| sACE | | | 1.02* | 1.00 – 1.03 | | | 1.02* | 1.00 – 1.03 | 1.01 | 1.00 – 1.03 | 1.00 | 0.97 – 1.05 | 1.00 | 0.97 – 1.05 | 1.01 | 1.00 – 1.03 |
| Age | | | | | 1.00 | 0.96 – 1.04 | 1.01 | 0.97 – 1.04 | | | | | 1.00 | 0.96 – 1.05 | 1.00 | 0.96 – 1.04 |
| Lymphocytes × sACE | | | | | | | | | | | 1.00 | 0.97 – 1.03 | 1.00 | 0.97 – 1.03 | | |
| Observations | 59 | | 59 | | 59 | | 59 | | 59 | | 59 | | 59 | | 59 | |
| $R^2$ Tjur | 0.203 | | 0.086 | | 0.203 | | 0.088 | | 0.222 | | 0.223 | | 0.224 | | 0.222 | |

* p<0.05 ** p<0.01 *** p<0.001