ORIGINAL ARTICLE

Can the laboratory affect the investigation and diagnosis of primary biliary cirrhosis?

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Background: Primary biliary cirrhosis (PBC) is an autoimmune liver disease characterised by the presence of various laboratory abnormalities but the precise role of laboratory staff in initiating clinical referral and subsequent biopsy is not clear.

Objective: To examine the impact of laboratory abnormalities in the investigation of PBC.

Methods: In a retrospective study of laboratory results over nine years from 1996, computer records were reviewed to identify how many referrals for biopsy were initiated and subsequent diagnoses made as a result of clinical signs, raised serum alkaline phosphatase activity (ALP), raised IgM concentration, or positive mitochondrial antibodies accompanied by a clinical comment from the laboratory suggesting further action.

Results: 22 diagnoses of PBC were confirmed by histopathology. Eleven had high ALP activity which had follow up tests initiated by the laboratory (mitochondrial antibodies or IgM or both) and a comment added suggesting further investigation into the possibility of PBC. Seven had abnormal liver antibodies and one had a high polyclonal IgM concentration which prompted the relevant follow on testing and comments. One had an earlier diagnosis made on serological/clinical grounds and the biopsy was a confirmatory measure. One had no liver related antibodies. One had a request by laboratory staff for follow on tests but these were not asked for in subsequent samples by the requesting clinician.

Conclusions: There is a positive role for laboratory staff in the diagnosis of PBC. Unexplained rises in ALP activity, positive mitochondrial antibodies, or raised IgM concentrations should be investigated more fully by laboratory staff and advice given to prompt a clinical referral for review and biopsy.

Primary biliary cirrhosis (PBC) is a rare disease characterised by inflammatory destruction of the small bile ducts within the liver. The cause of PBC is unknown, but because of the presence of characteristic mitochondrial autoantibodies, it is believed to have an autoimmune aetiology. There are abnormalities of the humoral and cellular immune systems (as shown by a raised IgM), with circulating autoantibodies. The disease has been associated with a variety of autoimmune mediated diseases (for example, autoimmune thyroiditis, keratoconjunctivitis sicca, scleroderma and its CREST variant). The major pathology involves the destruction of the small to medium bile ducts mediated by activated CD4 and CD8 lymphocytes, leading to progressive cholestasis and, in due course, end stage liver disease. As a result, chronic cholestasis is the prominent clinical and laboratory finding. About 90% of patients with PBC are female and the disease is diagnosed most commonly in patients between the ages of 40 and 60 years. The goals of treatment are to slow the progression rate of the disease and to alleviate the symptoms (such as pruritus, osteoporosis, and sicca syndrome). Liver transplantation appears to be the only life saving procedure.^{1 2}

The course of the disease is variable and an early diagnosis is desirable to identify individuals with rapidly progressing disease, to initiate adequate therapeutic measures, and to evaluate the need for liver transplantation. Diagnosis depends on a combination of clinical findings and laboratory results, with a liver biopsy confirming the diagnosis with its characteristic patterns of chronic, non-suppurative, destructive cholangitis of the small interlobular bile ducts, although there are suggestions that a liver biopsy is not always required to establish a diagnosis.³ There is an extensive literature on the presence of laboratory abnormalities (for example, alkaline phosphatase (ALP), mitochondrial

antibodies, and IgM concentrations) in PBC. One report suggests that 25% of PBC cases are incidentally diagnosed during a routine blood evaluation,⁴ but the precise role of the laboratory staff in initiating clinical referral and subsequent biopsy is less clear.

In this study we examined the impact of these laboratory factors in the investigation of this autoimmune liver disease.

METHODS

This was a retrospective study of clinical immunology, biochemistry, and histopathology results over a period of nine years from 1996. The study period was chosen to coincide with the introduction of a new laboratory information system; no biochemistry or immunology results were readily retrievable before 1996. Review of the computer records was undertaken to identify the route to diagnosis—that is, how many of the referrals for biopsy were initiated and subsequent diagnoses made as a result of clinical signs, raised ALP activity, raised IgM concentration, or positive mitochondrial antibodies, accompanied by a clinical comment from the laboratory suggesting further action.

Mitochondrial antibodies are detected in our laboratory as part of a liver related autoimmune panel consisting of antinuclear antibody, smooth muscle antibody, and mitochondrial antibody. We use an indirect immunofluorescence technique using rat liver as a substrate. ALP activity is measured on a routine automated analyser, using its ability to convert p-nitrophenyl phosphate (PNPP) to p-nitrophenol and produce a yellow colour. The rate of increase in absorbance from PNPP (colourless) to p-nitrophenol (colour) is directly proportional to the ALP activity. Total IgM was

Abbreviations: ALP, alkaline phosphatase activity; PBC, primary biliary cirrhosis

Table 1 Laboratory findings in 22 cases of primary biliary cirrhosis over nine years

Case	ALP activity (ref 30 to 95 IU/l)	Mitochondrial antibodies	IgM (ref 0.5 to 2.0 g/l)	Biopsy conclusions	Initial abnormal result
1	339	Positive	Not done	PBC	Mito
2	116	Weak positive	1.9	PBC	Mito
3	317	Strong	3.2	PBC	Mito
4	293	Moderate	4.1	PBC	Mito
5	171	Not done	1.7	PBC	Follow on tests requested but not done
6	1394	Weak	5.7	Active hepatitis/PBC	Mito
7	580	Moderate	Not done	PBC	Mito with previous high ALP
8	187	Moderate	2.2	PBC	Mito
9	Previous diagnosis of PBC; confirmatory biopsy undertaken				
10	171	Negative	Not done	PBC	ALP
11	280	Moderate	3.0	Chronic hepatitis and PBC	ALP
12 (M)	1604	Moderate	4.8	PBC	ALP
13	167	Strong	3.2	PBC	ALP
14	160	Strong	17.8	PBC	IgM
15	604	Strong	4.3	PBC	ALP
16	60	Moderate	0.9	CAH/PBC overlap	Mito
17	285	Moderate	1.9	PBC	ALP
18	174	Moderate	4.3	CAH/PBC	ALP
19	2248	Moderate	8.8	PBC	ALP
20	454	Moderate	8.6	PBC	ALP
21	324	Moderate	7.2	PBC	ALP
22	120	Moderate	2.2	PBC	Mito

ALP, alkaline phosphatase; CAH, chronic active hepatitis; M, male; mito, mitochondrial antibodies; PBC, primary biliary cirrhosis; ref, reference values.

estimated using a nephelometer. As this study spanned nine years, with many equipment changes during this period, the methods rather than the equipment are itemised here.

Liver biopsy samples were processed into paraffin blocks, sections cut at 3 µm thickness, and stained with haematoxylin and eosin, periodic acid Schiff (PAS), PAS with diastase, untuned reticulin, Van Gieson, congo red, orcein, Perls, and Gomori's aldehyde fuchsin.

In requesting further investigations, the selection criteria employed by the laboratory staff involved using their professional judgement to interpret a combination of patient age, sex, which consultant was involved in looking after the patient, current and previous clinical data, and any previous laboratory results. Unexplained ALP activities greater than 150 IU/l (reference values 30 to 95) were investigated by testing for mitochondrial antibodies and, if positive, by estimating the total IgM concentration. In the case of the chance finding of a moderately or strongly positive test for mitochondrial antibody, ALP activity was estimated and, if raised, the IgM concentration measured. Finally, an unexplained rise in IgM prompted an estimation of ALP activity and, if raised, a mitochondrial antibody assay. This algorithm was aided by the fact that this is a joint clinical biochemistry and immunology laboratory, with easy access to samples and methods.

RESULTS

A computer search to identify all histopathological diagnoses of PBC was undertaken and 22 such diagnoses were confirmed by histopathology during the study period. Twenty one were in women, with ages ranging from 39 to 69 years, and a mean of 55.9 years at the time of liver biopsy. The sole man was aged 59 years.

Table 1 shows the results of 22 patients diagnosed with PBC by histology in the preceding nine years in our hospital. Eighteen of these were assessed clinically and had subsequent liver biopsies as a result of the further examination of laboratory data.

Eleven patients had a high ALP activity which initiated follow up tests by the laboratory (mitochondrial antibodies or IgM, or both) and in each case a comment was added suggesting further investigation into the possibility of PBC. Seven patients had an abnormal liver autoimmune profile

with positive mitochondrial antibodies which prompted follow on testing and comments. One patient had an abnormally high polyclonal IgM concentration which prompted the relevant follow on testing and comments. One patient had an earlier diagnosis made on serological and clinical grounds and the biopsy was a confirmatory measure. One patient had no liver related antibodies and was classified as mitochondrial antibody negative PBC. One patient from early in the study (1997) had follow on tests requested by laboratory staff but these were not asked for in subsequent samples by the requesting clinician. In this case, a diagnosis of chronic active hepatitis had been suggested but there was nothing in the immunoglobulin profile to support this. We had suggested a liver autoimmune panel would be useful but this was not carried out.

DISCUSSION

This study has demonstrated a positive role for clinical laboratory staff in the diagnosis of PBC. Clinical biochemists and immunologists cooperate with clinicians who require consultation, advice, and information for the appropriate use of biochemical or immunological markers, but one of the debates in laboratory circles is whether or not the addition of comments to aid interpretation of results has any effect on patient care. This study confirms that the addition of a comment suggesting clinical referral for further investigation for primary biliary cirrhosis is heeded by the requesting clinician, even though the condition may not have been in the original differential diagnosis. It also shows the value of using professional judgement to undertake extra tests following an initial abnormal result that should be investigated more fully.

The 22 PBC diagnoses made in the study period is very similar to other north European epidemiological studies—for example, Boberg *et al* reported 21 such diagnoses in a population of 130 000 over a 10 year period.⁵ Our 22 cases clearly do not include any "diagnoses" made on clinical or other grounds and without recourse to liver biopsy. We chose to concentrate on those patients for whom there was the gold standard of a biopsy diagnosis.

Seven of these diagnoses were made following the initial finding of a positive test for mitochondrial antibody. These can be non-specific in nature, may be found in many

rheumatic and infectious conditions, and are not diagnostic of PBC. Therefore, before resources are used in investigating every positive mitochondrial antibody result, we should be guided by the other physical, clinical, and laboratory features of the patient and an assessment made of the likely relevance of the antibody result. There is, however, one published report suggesting that before the advent of any clinical or biomedical indications, individuals positive for E2 subunits of pyruvate dehydrogenase complex and 2-oxoglutarate dehydrogenase complex mitochondrial antibodies do have early PBC.⁶ Thus, if a positive mitochondrial antibody test is found, particularly in a middle aged woman, then follow on tests such as total IgM and alkaline phosphatase should be undertaken. Similarly, a raised IgM in a middle aged woman should be investigated more fully. Only one of the patients had a raised IgM as the initial finding. Raised IgM concentrations can be found in PBC but also in acute viral infections, lymphoma with the presence of monomeric IgM, and protozoal parasitic infections. The majority of the patients had a raised ALP activity at the time of presentation. Raised ALP activities are very common, especially in a middle aged or elderly population. This test is likely to be a sensitive but non-specific marker for PBC. ALP activity will be raised in bone disease—for example, osteomalacia, Paget's disease, or secondary bony deposits from a distant primary carcinoma. It may also be raised in intrahepatic or extrahepatic cholestasis, any space occupying lesion, hepatic infiltration, or other malignancy. However, if no obvious cause can be found for an ALP activity of 150 IU/L or more (or 1.5 times the upper limit of normal for the laboratory), then follow on tests for liver autoantibodies appear justified. If these are present, this could be followed up with an estimation of total IgM.

This was a nine year retrospective study that concentrated on the benefits to patients rather than the costs. These "costs" might include the financial and clinical implications. They will include the effect on the patient of inappropriately repeated blood tests, as well as the costs to the laboratory in doing extra tests. It would be interesting, for example, to know how many times the extra tests were done over the nine year period and how many gave normal results. Our study, however, was not designed to answer that question, which is the subject of an ongoing prospective study. As the decision to involve invasive tests such as liver biopsy is taken in the light of the clinical scenario as well as the laboratory findings, it is unlikely that inappropriate liver biopsies were done. The various costs involved could only be quantified by a long term prospective study, but that does not detract from the main message of this study which is that the laboratory has a role to play in the investigation of such patients.

Inappropriate use of laboratory services unjustifiably increases health care costs, may even harm patients, and perpetuates the notion that laboratory testing is a commodity. Improvement in the appropriateness of laboratory testing can be achieved by developing a closer relation with the requesting physicians and by stressing the role of laboratory specialists in providing clinical advice for the selection of laboratory tests, and the interpretation of their results, thus leading to more satisfactory clinical outcomes. There is published work showing that the requesting of extra tests by laboratory staff, and interpretive comments added to laboratory results, can have a marked impact on patient management. This is an area important enough to have its own external quality control schemes.^{7,8} Kilpatrick has provided objective data that the addition of interpretive comments had a direct effect on management of patients undergoing thyroxine replacement,⁹ and others have

Take home messages

- There is a positive role for laboratory staff in the diagnosis of primary biliary cirrhosis.
- Unexplained rises in alkaline phosphatase activity, positive mitochondrial antibodies, or raised IgM concentrations should be investigated more fully by laboratory staff and advice given to prompt a clinical referral for review and biopsy.

recommended that the practice should be limited to those staff with clear expertise.¹⁰ Our own work has demonstrated the profound effect that comments attached to positive gut related antibody results has on the subsequent referral for small bowel biopsy.¹¹ However, this practice does have ethical considerations if tests seemingly unrelated to the presenting symptoms are added by laboratory staff. Our experiences¹² and those of others¹³ are that the laboratory should play an active role in this process. It is, of course, very likely that the diagnoses of PBC would have been made regardless of the laboratory's involvement in some of the cases illustrated here. For example, patient 19 had a grossly elevated ALP and this would have been noted by the requesting clinician and presumably acted upon. However, some of the laboratory immunology and biochemistry results are more subtle and these data have shown that our involvement has hastened the diagnosis in many cases. This study also shows the benefits of integrated departments where different disciplines have easy access to samples and one another's results. As Kerr observed recently, a patient with liver disease does not care that the liver enzymes are measured in one department and the autoantibodies in another.¹⁴

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