Azathioprine induced serious portal hypertension : a case series of three IBD patients and review of the literature

D. Dooremont[1,2], J. Decaestecker[1], D. De Wulf[1], G. Ghillebert[1], H. Van Vlierberghe[2], J. Van Dorpe[3], F. Baert[1]

(1) Dept of Gastroenterology at H.-Hart ziekenhuis Roeselare- Menen VZW, Roeselare, Belgium ; (2) Dept of Gastroenterology & Hepatology, Ghent University Hospital, Gent, Belgium ; (3) Dept of Anatomopathology at H.-Hart ziekenhuis Roeselare-Menen VZW, Roeselare, Belgium.

Abstract

We report 3 male IBD patients (2 Crohn's Disease, 1 Ulcerative Colitis) developing thrombocytopenia and splenomegaly on azathioprine treatment. All patients were diagnosed with significant portal hypertension due to histological proven nodular regenerative hyperplasia (NRH) of the liver. In two of three patients, liver function tests remained completely normal.

In addition we provide a short literature review of azathioprine induced NRH covering etiology, imaging, pathology, prognosis and treatment. (Acta gastroenterol. belg., **2013**, 76, **342-346**).

Key words : case series, IBD, azathioprine, nodular regenerative hyperplasia, portal hypertension.

Introduction

Azathioprine was first introduced into clinical practice by Sir Roy Calne, the British pioneer in transplantation (1). Since its introduction in 1961 worldwide it has helped many patients. Following its use to prevent rejection in organ transplantation it is now registered or indicated in a variety of auto-immune mediated diseases (including rheumatoid arthritis, pemphigus, multiple sclerosis, inflammatory bowel disease (Crohn's disease and ulcerative colitis) and auto-immune hepatitis). Because of its side effect profile, genotyping for thiopurine methyltransferase (TPMT) and/or routine biochemistry testing is mandatory upon starting azathioprine/6 mercapto-purine to screen for the most common side effects including bone marrow-toxicity (leucopenia, thrombocytopenia, pancytopenia), elevated liver enzymes and pancreatitis. Even during maintenance therapy, screening is recommended. In the following case reports we want to draw attention to another insidiously occurring, less frequent but potentially fatal side effect.

Case reports

First case

A 37-year old male with a past history of asthma bronchiale developed ulcerative colitis in 2004. In May 2005 he had a severe flare of pancolitis for which azathioprine was started at a dose of 2 mg/kg without hematological problems. In December 2005 he was diagnosed with a severe pancytopenia after being proscribed allopurinol for a bout of gout. Because of persistent pancytopenia despite stopping allopurinol he underwent TPMT genotyping revealing a heterozygous mutation in the thiopurine methyltransferase-enzyme. Azathioprine was stopped and switched to methotrexate with no effect on the ulcerative colitis. In 2007 a new attempt to give azathioprine followed by pancytopenia again led to definitively discontinuation of azathioprine. His colitis went into remission with the use of mesalazine (5-ASA) monotherapy. In total he received during 6 months azathioprine in 2005 and during 3 months in 2007.

However in the following years the patient had a persistent thrombocytopenia (ranging between 59.000 and 72.000/mm^3), for which hematologic investigations were performed. All tests (including bone marrow examinations) revealed no apparent cause.

In January 2011 the patient was admitted to the emergency room of our hospital after an episode of massive hematemesis with hypotension.

After resuscitation and intubation an urgent oesophagogastroscopy was performed. This revealed grade II oesophageal varices and an active bleeding varix in the gastric fundus. Hemostasis could be achieved by injection of histo-acryl.

The blood results at admission are presented in table 1. Other testing obtained included normal iron parameters, normal copper and ceruloplasmine, normal alfa1-antitrypsine, normal alfa-foetoprotein, normal autoimmune serology, no signs of viral hepatitis (hepatitis B,C,E, EBV). An ultrasound of the abdomen revealed a rough aspect of the liver and splenomegaly (140 mm). In the further work-out we performed a CT-scan which showed sequellae of sclerotherapy in the gastric fundic varices, distension of the portal and splenic veins, steatosis of the liver without arguments for liver cirrhosis and neither for portal vein thrombosis. An MRI scan of the liver showed no new findings, other than a rough aspect of the liver parenchyma, identical signs of portal hypertension and a thickened gallbladder wall.

Correspondence to : Diederik Dooremont, M.D., Paddestraat 41, 9620 Velzeke-Ruddershove, Belgium. E-mail : diederikdooremont@yahoo.com

Submission date : 28/08/2012
Acceptance date : 21/09/2012

Table 1. — **Laboratory tests at presentation with NRH of our 3 patients**

	Pt 1	Pt 2	Pt 3	Normal Values
Hemoglobine	9.1	12.1	14.6	13.3-16.7 g/dl
Leucocytes	4050	3510	5590	3700-9500/mm^3
%Neutrophils	67	69	75	46-64%
%Lymphocytes	23	21.7	11	27-37%
Platelets	65	57	109	150-450 × 10^3/mm^3
Total bilirubine	0.97	0.82	2.5	0.00-1.20 mg/dl
AST	18	26	77	10-50 U/L
ALT	21	20	102	10-50 U/L
Alkal phospatase	35	65		53-128 U/L
G-GT	27	16	246	11-49 U/L

A biopsy of the liver was performed which revealed discrete architectural alterations. Very rare incomplete fine portoportal septa were seen in this biopsy and there was minimal pericellular fibrosis. Liver sinusoids showed slight dilatation. A reticulin stain did not show nodular regeneration. Portal and paraportal small dilated thin-walled vessels were an obvious feature in this biopsy. In addition, arterioles showed a slightly thickened tunica media. Bile duct lesions, suggestive for PBC or PSC, were not present. There was no portal or lobular inflammatory infiltrate. Hepatocytes showed minimal anisonucleosis, some centrolobular lipofuscin accumulation, and very rare glycogenated nuclei, but no steatosis, Mallory bodies or apoptosis. The subtle alterations in this biopsy were suggestive for incomplete septal cirrhosis.

Second case

A 44-year old male patient with a history of severe Crohn's disease with need for several surgical interventions and resections was referred in 2010 to our hematology department because of splenomegaly and persistent thrombocytopenia (57 000/mm^3). Seven years before he received a 2 month course of azathioprine, complicated by a pancytopenia. Before this period we have no data over the use of azathioprine. This lead to a definite discontinuation of the azathioprine treatment. Subsequently leucopenia and anemia recovered slowly, however thrombocytopenia persisted. In 2010 a routine ultrasound of the abdomen showed a splenomegaly (150 mm) and a normal image of the liver. The hematologic evaluation, including bone marrow examination, was normal. The liver enzymes were completely normal (Table 1). Subsequently a CT-scan was performed which showed an image of cirrhosis with portal hypertension. The gastroscopy showed grade 3 oesophageal varices. The liver biopsy showed architectural alterations that were slightly more pronounced than seen in the first patient. In addition to slight periportal fibrosis with formation of rare fine portoportal septa, some sinusoidal dilatation, and minimal centrolobular pericellular fibrosis, there was vague nodularity, best seen on a reticulin stain, suggesting early nodular regeneration (Fig. 1). Some small paraportal small dilated thin-walled vessels were present. Hepatocytes showed minimal anisonucleosis and rare glycogenated nuclei, but no steatosis. Inflammation or bile duct lesions were not present.

Subsequently obtained TPMT genotyping showed that he had heterozygosity for TPMT*3A, suggestive for an intermediate enzymatic activity of TPMT.

Third case

A 34-year old male patient with the diagnosis of Crohn's disease since 1997 was referred to our department because of a sudden increase in liver enzymes and the development of thrombocytopenia, under a maintenance treatment with azathioprine 150 mg daily. In 1998 he underwent a partial intestinal resection. He received azathioprine in 1998 during one year and was restarted on azathioprine from 2005 until September of 2011. In total he was treated for at least seven years with 2 mg/kg azathioprine. In November 2010 when the last testing was performed, his blood results (including liver tests and platelet counts) were normal. However in September 2011 the liver enzymes were disturbed including a SGOT/AST of 77 U/L, SGPT/ALT 102 U/L and gamma GT 246 U/L. The total bilirubin raised to 2,5 mg/dl (but the patient is known with a Gilbert syndrome (unconjugated hyperbilirubinemia). In addition the patient had a low platelet count of 109000/mm^3 (Table 1). The ultrasound showed a homogenous liver with a slightly enlarged caudate lobe and a splenomegaly (150 mm). One month after stopping azathioprine the abnormalities remained unchanged. A liver biopsy was performed showing an altered architecture. Some fine portoportal and portocentral septa, delicate periportal and perivenular fibrosis, and dilated small thin-walled portal vessels were present. Reticulin stain demonstrated a vague nodularity and suggested nodular regeneration. Hepatocytes showed anisonucleosis, some glycogenated nuclei, and minimal macrovesicular steatosis. There was a minimal portal and lobular inflammatory infiltrate consisting of lymphocytes and very rare plasmocytes. Bile duct lesions were absent. In summary, this biopsy showed features of early nodular regenerative hyperplasia, slight fibrosis, inconspicuous macrovesicular steatosis and minimal inflammation. Gastroscopy showed grade I oesophageal varices. In this patient we could not demonstrate a mutation in the TPMT gene.

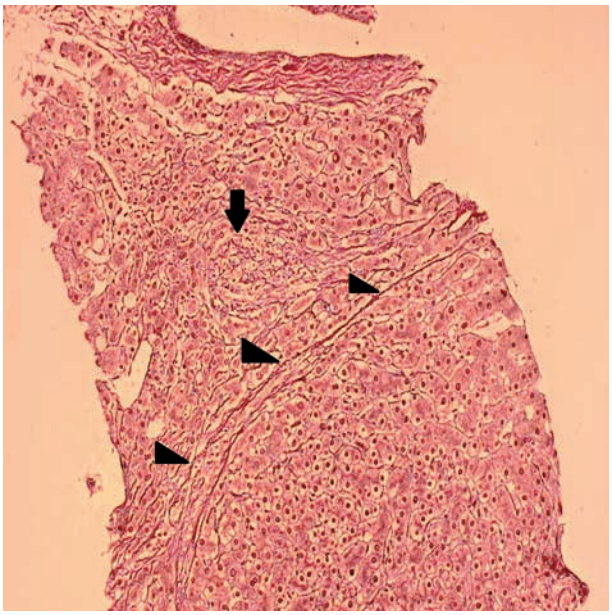

Fig. 1. — Liver biopsy from patient 2 showing subtle architectural alterations (reticulin stain ; ×100). A thin septum is indicated by arrowheads. The upper field of the picture shows a dilated thin-walled vessel in a portal tract. A small portal tract with minimal periportal fibrosis is indicated by an arrow. There is only vague nodularity.

Discussion

We report three cases of azathioprine induced liver disease with significant portal hypertension. Liver biopsies from these patients showed fine portoportal septa, minimal pericellular fibrosis, some sinusoidal dilatation, vague or indistinct nodularity, and portal and paraportal dilated thin-walled vessels. These abnormalities are features of incomplete septal cirrhosis, which is a subtle anatomical type of cirrhosis. Incomplete septal cirrhosis is difficult to diagnose and shows a relationship and overlap with other forms of non-cirrhotic hypertension : nodular regenerative hyperplasia and hepatoportal sclerosis (non-cirrhotic portal fibrosis). Collectively, incomplete septal cirrhosis, nodular regenerative hyperplasia and non-cirrhotic portal fibrosis are thought to be a spectrum of diseases with a common pathogenesis related to abnormalities in vascular supply. Obliterative portal venopathy is postulated to be the cause of these interrelate disorders (2). There are a wide range of conditions or diseases that can lead to the development of NRH. The most important are haematologic, auto-immune and lymphoproliverative syndromes and certain drugs (including chemotherapeutic agents, purine analogs and antiretroviral agents (2,3). Also ulcerative colitis and Crohn's disease in itself can lead to nodular regenerative hyperplasia, without the use of purine analogs[2]. In our cases, azathioprine is the most likely cause of liver damage, although we cannot exclude the role of IBD in the pathogenesis. Especially in our third patient, the histology showed mild inflammation and macrovesicular steatosis, not typically seen in NRH, suggesting a more multifactorial etiology of the liver injury.

The pathophysiology of NRH remains unclear. Purine analogs, azathioprine (AZA), 6-mercaptopurine (6-MP) and 6-thioguanine nucleotides (6-TGN) are widely used in the treatment of inflammatory bowel disease.

Azathioprine and 6-MP are both inactive pro-drugs. Azathioprine is converted to 6-MP, which is then metabolized via three different routes. The 6-TGN has the most important immune modulatory effects of the thiopurines but is also associated with bone marrow suppression at high concentrations (4). The concomitant use of allopurinol results in an inhibition of the xanthine oxidase which itself leads to an increase in the 6-TGN concentrations, resulting in greater immunosuppression and risk of leukopenia (5). Accordingly we would also expect a rise in the production of 6-MMP when the xanthine oxidase is inhibited. In contrary the opposite has been noted in clinical studies, namely a decrease in production of 6-MMP (6,7). The mechanism for this reduction is unknown. The 6-methylmercaptopurine nucleotides (6-MMP) are associated with hepatotoxicity, although other metabolites also contribute (4).

It is well known that the use of 6-TGN (6-thioguanine) is associated with an increased risk of serious hepatotoxicity (8). It is very likely that our first patient was exposed to massive concentrations of 6-thioguanines because of the accidental use of allopurinol and the fact that he was heterozygous for mutations of thiopurine methyltransferase (TPMT). The same is true for our second patient because of his intermediate activity of TPMT. Both patients developed a quick and severe pancytopenia, but the hint towards NRH was the persistent thrombytopenia suggesting an early onset of the liver injury. Accumulation of toxic metabolites may be a factor, as in 2 of our three patients a mutation was shown in the TPMT[2]. On the contrary our third patient, with normal TPMT genotyping was probably exposed to more normal doses during at least 7 years before developing liver function abnormalities and thrombocytopenia.

In the past 6-TGN treatment was used in an attempt to prevent the side effects and optimize the effect of azathioprine and 6-MP. Initially promising results of 6-TG in Crohn's disease were rapidly followed by an alarming report of a high incidence of NRH (8). Of 111 patients treated with 6-TGN, 26% presented with biological abnormalities (thrombocytopenia and abnormal functional liver tests) suggestive for NRH. A liver biopsy confirmed this diagnosis in 76% of these cases and in 33% of the totally asymptomatic patients who had agreed to undergo biopsy (8). As a result of this study, 6-TG treatment was suspended in most countries.

Vernier-Massouille *et al*. investigated the prevalence and risk factors for developing NRH in patients with inflammatory bowel disease treated with azathioprine. The cumulative risk of developing NRH was estimated at 0,50% at five years and 1,25% at 10 years (9). This rate is very similar to that of a more recent study by Seksik,

reporting a cumulative estimated risk of 1,28%, 10 years after starting treatment (10). These figures are likely an underestimation of the true prevalence as, unlike in our first patient, NRH often remains asymptomatic. Only patients presenting with clinical symptoms or biological abnormalities were reported and no screening procedures were systematically undertaken.

The most important risk factors for developing azathioprine / 6-mercaptopurine induced NRH are male gender and a history of small bowel resection (> 50 cm). In this group of male patients who had experienced extensive small bowel resection the cumulative incidence of NRH reaches about 10% after 10 years (9,10). This is also evident from this case series, all of our patients are male and two of them had resections of small bowel in the past. So in this group of patients more precaution is necessary and perhaps screening becomes cost-effective.

Blood analysis may show a mild elevation in the transaminases. However in most of the cases the transaminases remain normal since there is no or very little necrosis of hepatocytes. Elevation of gamma-glutamyl transpeptidase and alkaline phosphatase due to cholestasis is observed in 10-15% of the patients (11). In contrast to cirrhosis, the liver function remains often normal with a normal serum bilirubin, albumin and prothrombin time, as illustrated in our first two cases (11). In our third case a sudden rise in liver enzymes appeared after several years of treatment which is rare in NRH. This patient had also a rise in (indirect) bilirubine but was known with a Gilbert syndrome. Clinical symptoms can occur when the portal hypertension evolves (9,10,11,12). The most frequent signs and symptoms are variceal bleeding, ascites, edema of the lower limbs, splenomegaly and hence secondary thrombocytopenia (2).

Imaging modalities have a poor sensitivity and specificity for NRH. On ultrasound, regenerative hyperplastic nodules are usually not visible due to their small size (1-3 mm) or isoechogenicity (2). Caturelli reports 4 cases of proven pathology NRH with small, round isoechoic lesions with a thin hyperechoic rim, resembling the ring-shaped coral-reef configuration known as an atoll as a characteristic ultrasound finding (3). These lesions identified on ultrasound represent only the larger nodules, diameters up to 15 mm have been reported (3). On computed tomography (CT), regenerative nodules remain isodense or hypodense in both arterial and portal venous phases, distinguishing NRH from focal nodular hyperplasia and adenomas. The use of magnetic resonance in the diagnosis of NRH remains still controversial. NRH lesions appear hyperintense on T1-weighted images and iso- or hypointense on T2-weighted images, with a sensitivity and specificity of 70%-80% when using gadolinium contrast. Others found more disappointing results (2,13). Also the use of MRI with contrast (gadolinium-chelate and ferucarbotran) is unreliable in diagnosing NRH in this study (13). All these findings are similar in our case reports, neither ultrasound, neither CT, nor MRI could diagnose NRH. Imaging was only capable of diagnosing the portal hypertension.

Laharie evaluated the usefulness of noninvasive fibrosis tests like FibroTest and FibroScan. In case of portal hypertension suggesting NRH, whatever the level of associated fibrosis on biopsy, FibroTest and FibroScan cannot distinguish NRH from cirrhosis and should be interpreted carefully (13).

A definite diagnosis of NRH is made at histopathology of the liver demonstrating regenerative nodules without parietal thickening of portal venules and no or mild portal fibrosis on reticulin staining. Fibrosis is minimal or absent between one nodule and another, and this element distinguishes NRH from cirrhosis (3). Dilated sinusoids and thrombosed portal vein radicles are occasionally present (3). In all three patients minimal fibrosis and some sinusoidal dilatation was seen, in the last two patients there was vague nodularity seen on the reticulin staining, suggesting NRH. Like mentioned earlier, the mild inflammation and steatosis in the third patient suggests a more multifactorial etiology of the liver injury.

The pathophysiology of NRH is poorly understood. The mostly widely accepted view is that NRH is the result of obliterative vascular disease. There is a hypothesis that heterogenous perfusion of the hepatic parenchyma leads to hepatocytic atrophy in hypoperfused areas and compensatory hyperplasia in those which remain normally vascularized (9,14). NRH associated with azathioprine might be secondary not to portal venule lesions but rather to sinusoidal lesions, through depletion of glutathione (9).

Generally, the prognosis of NRH is better than that of chronic liver disease and is related to the complications of portal hypertension and the severity of the underlying disease. Nodular regenerative hyperplasia can remain asymptomatic for a long time. In most cases it is a slowly progressive disease. It is unknown whether NRH can progress after discontinuing the putative drug. However NRH is probably not reversible, even after stopping the treatment with azathioprine. Although there is one case report by Seiderer of an azathioprine induced NRH, proven on MRI imaging and by liver biopsy where after twelve months after stopping azathioprine the MRI image returned to normal (12). However a liver biopsy was declined by the patient.

NRH is a condition that has been associated with liver cell dysplasia, a known premalignant situation. Hence these patients are at risk to develop hepatocellular carcinoma (HCC) in the absence of cirrhosis. Therefore screening for hepatocellular carcinoma (HCC), by ultrasound and alfa-foetoprotein has been recommended (15).

Our small case series coming from a single regional center in a short period of time indicates the condition is not so rare. Physicians prescribing azathioprine should be aware of NRH. In the absence of abnormal liver tests, thrombocytopenia and splenomegaly may be interpreted as a hematological or lymphoproliferative condition.

Indeed two of our three patients underwent a bone marrow examination before the diagnosis of NRH was raised.

Conclusion

Nodular regenerative hyperplasia (NRH) can occur as a rare adverse event of thiopurine treatment. Physicians using azathioprine should consider NRH in their differential diagnosis when patients develop signs or symptoms of portal hypertension like unexplained thrombocytopenia, splenomegaly, oesophageal or gastric varices or frank bleeding. Normal liver enzymes do not exclude NRH. Male patients and patients with extensive bowel resections are at increased risk. In addition we suggest that patients with higher 6 TGN metabolites (such as TPMT mutated and or allopurinol co-treated patients) are also at increased risk. A suspected diagnosis should be confirmed by liver biopsy. The treatment consists of stopping the causative drug(s), preventing and treating complications due to the portal hypertension. Liver synthesis usually remains normal however patients with NRH are advised to undergo screening for dysplasia and HCC.

References

1. CALNE R. Essay : History of transplantation. *The Lancet*, 2006, **368** (Suppl 1) : S51-S52.
2. HARTLEB M., GUTKOWSKI K., MILKIEWICZ P. Nodular regenerative hyperplasia : evolving concepts on underdiagnosed cause of portal hypertension. *World. J. Gastroenterol.*, 2011, **17** (11) : 1400-1409.
3. CATURELLI E., GHITTONI G., RANALLI T.V., GOMES V.V. Nodular regenerative hyperplasia of the liver : coral atoll-like lesions on ultrasound are characteristic in predisposed patients. *Brit. J. Radiol.*, 2011, **84** : e129-e134.
4. GEARRY R.B., DAY A.S., BARCLAY M.L., LEONG R.W.L, SPARROW M.P. Azathioprine and allopurinol : A two-edged interaction. *J. Gastroenterology. Hepatol.*, 2010, **25** : 653-655.
5. http://www.uptodate.com/contents/6-mercaptopurine-6-mp-metabolite-monitoring-and-TPMT-testing-in-the-treatment-of-inflammatory-bowel-disease-with-6-mp-or-azathioprine
6. SPARROW M.P., HANDE S.A., FRIEDMAN S., CAO D., HANAUER S.B. Effect of allopurinol on clinical outcomes in inflammatory bowel disease nonresponders to azathioprine or 6-mercaptopurine. *Clin. Gastroenterol. Hepatol.*, 2007, **5** (2) : 209-214.
7. SPARROW M.P., HANDE S.A., FRIEDMAN S. *et al*. Allopurinol safely and effectively optimizes tioguanine metabolites in inflammatory bowel disease patients not responding to azathioprine and mercaptopurine. *Aliment. Pharmacol. Ther.*, 2005, **22** : 441-446.
8. DUBINSKY M.C., VASILIAUSKAS E.A., SINGH H. *et al*. 6-thioguanine can cause serious liver injury in inflammatory bowel disease patients. *Gastrolenterology*, 2003, **125** : 298-303.
9. VERNIER-MASSOUILLE G., COSNES J., LEMANN M. *et al*. Nodular regenerative hyperplasia in patients with inflammatory bowel disease treated with azathioprine. *Gut*, 2007, **56** : 1404-1409.
10. SEKSIK P., MARY J-Y., BEAUGERIE L. *et al*. Incidence of nodular regenerative hyperplasia in inflammatory bowel disease patients treated with azathioprine. *Inflamm. Bowel. Dis.*, 2011, **17** : 565-572.
11. ARVANITAKI M., ADLER M. Nodular regenerative hyperplasia of the liver. A review of 14 cases. *Hepatogastrol.*, 2001, **48** (41) : 1425-1429.
12. SEIDERER J., ZECH C.J., DIEBOLD J. *et al*. Nodular regenerative hyperplasia : a reversible entity associated with azathioprine therapy. *Eur. J. Gastroenterol. Hepatol.*, 2006, **18** : 553-555.
13. LAHARIE D., VERGNIOL J., BIOULAC-SAGE P. *et al*. Usefulness of noninvasive tests in nodular regenerative hyperplasia of the liver. *Eur. J. Gastroenterol. Hepatol.*, 2010, **22** : 487-493.
14. IBAROLLA C., COLINA F. Clinicopathological features of nine cases of non-cirrhotic portal hypertension : current definitions and criteria are inadequate. *Histopathology*, 2003, **42** : 251-264.
15. NZEAKO U.C., GOODMAN Z.D., ISHAK K.G. Hepatocellular carcinoma and nodular regenerative hyperplasia : possible pathogenetic relationship. *Am. J. Gastroenterol.*, 1996 May, **91** (5) : 879-884.