

# Methotrexate-associated liver toxicity in a patient with breast cancer: case report and literature review

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## ABSTRACT

A patient with breast cancer developed severe asthenia, accompanied with progressively increasing transaminases, during adjuvant chemotherapy with CMF (cyclophosphamide, methotrexate and 5-fluorouracil). Additional blood tests and imaging were negative. A liver biopsy revealed a grade II toxic hepatitis. Because methotrexate was suspected to be the cause of the hepatotoxicity, the administration of this drug was stopped and mitoxantrone was given instead. A recovery of clinical symptoms and normalisation of the liver function tests was observed afterwards. In that sense, mitoxantrone appears to be a valuable alternative to methotrexate in cases of hepatotoxicity in patients with breast cancer. An overview of the literature regarding methotrexate hepatotoxicity is presented.

## INTRODUCTION

Breast cancer is the most common malignancy among women in Europe. Depending on the stage, the treatment consists of local surgery (tumourectomy or mastectomy with axillary lymph node dissection) with or without post-operative radiotherapy and adjuvant systemic therapies. Several chemotherapeutic agents are used in the adjuvant setting when treating patients with breast cancer. Common chemotherapy combinations are CMF (cyclophosphamide, methotrexate, 5-fluorouracil), AC (doxorubicin, cyclophosphamide), FAC (5-fluorouracil, doxorubicin, cyclophosphamide) and FEC (5-fluorouracil, epirubicin, cyclophosphamide). The most frequently observed toxicities with these treatment schedules are

myelosuppression, nausea and vomiting, stomatitis and diarrhoea. The anthracycline-based regimens in particular may induce more severe hair loss and cardiotoxicity. We describe a patient with breast cancer who developed a toxic hepatitis due to methotrexate within the context of an adjuvant therapy with CMF and give a literature review on methotrexate-induced liver toxicity.

## CASE

A 60-year-old woman with a history of asymptomatic cholecystolithiasis complained of a mass in the left breast. She first detected the mass several weeks before admission to the hospital. On clinical examination, a tumour of two centimetres in diameter was indeed palpated in the superolateral quadrant of the left breast, without any suspicion of involved axillary lymph nodes. Mammography confirmed the presence of a suspicious lesion in the same quadrant. Pre-treatment evaluation consisted of a chest X-ray, bone scan and ultrasound examination of the liver and was considered negative. The tumour marker (CA 15.3) at baseline was within normal limits, as was the kidney function. Before treatment, aspartate aminotransferase (AST) was 25 U/l (normal values 5-40 U/l) and alanine aminotransferase (ALT) 37 U/l (7-56 U/l). Alkaline phosphatases (AP) at baseline were 49 U/l (36-95 U/l), lactate dehydrogenase (LDH) was 443 U/l (313-618 U/l). Gamma-glutamylpeptidase ( $\gamma$ -GT) was slightly increased to 36 U/l (11-29 U/l). No hypoalbuminaemia was present. A tumourectomy and axillary lymph node dissection were

performed. The pathological examination showed a poorly differentiated ductal adenocarcinoma of 1.7 cm in greatest dimension. One of the 17 removed axillary lymph nodes was involved. Oestrogen and progesterone receptors were absent. Therefore, the diagnosis of a pT<sub>1c</sub>pN<sub>1bi</sub>M<sub>0</sub> breast cancer was made.

Postoperative treatment consisted of irradiation of the breast, the left internal mammary chain and the supraclavicular nodes (50 Gy in 25 fractions with an additional boost on the tumour bed). The planned adjuvant chemotherapy consisted of cyclophosphamide (CTX) 600 mg/m<sup>2</sup>/day, methotrexate (MTX) 40 mg/m<sup>2</sup>/day, and 5-fluorouracil (5-FU) 600 mg/m<sup>2</sup>/day on day 1 and day 8, given every four weeks for six cycles. Corticosteroids were administered before each new chemotherapy cycle.

After two cycles of chemotherapy, the patient complained of asthenia. She stayed in bed several hours a day. There was no fever, nausea, vomiting, abdominal pain, cough or weight loss. Her appetite was normal; oral food intake remained possible. On clinical examination, her general condition was good and no abnormalities were detected. The patient was only taking low doses of benzodiazepines. Blood examination revealed slightly elevated transaminases: AST of 45 U/l and ALT of 64 U/l. The other liver function tests, including  $\gamma$ -GT, were within normal limits. Two more

cycles of CMF were administered. A minor weight loss of two kilograms (without nausea or vomiting) and a further deterioration of the liver function tests accompanied them. AST rose to 88 U/l, ALT increased to a value of 98 U/l (figure 1). AP, LDH and  $\gamma$ -GT increased to 108 U/l, 915 U/l and 83 U/l, respectively. Bilirubin levels remained within normal limits. Serum levels of creatinine were normal. A mild hypoalbuminaemia developed nevertheless.

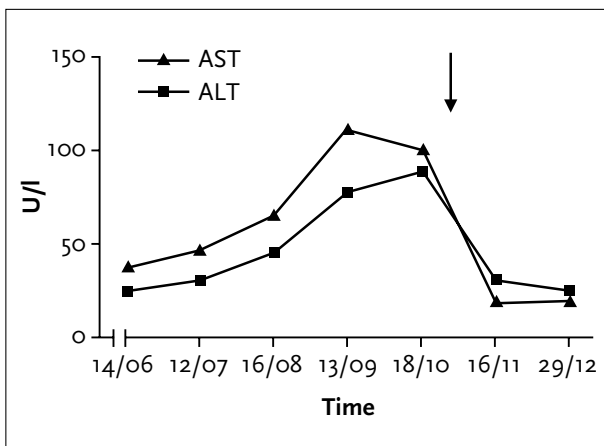
Albumin decreased to a minimum value of 2.6 g/dl (3.2-5.0 g/dl). The tumour marker (CA 15.3) increased to 51.1 U/ml (nl <35).

An ultrasound examination of the abdomen only revealed a small cyst in liver segment 7, without other changes.

There were no signs of liver metastases. This was confirmed by triphasic CT scanning.

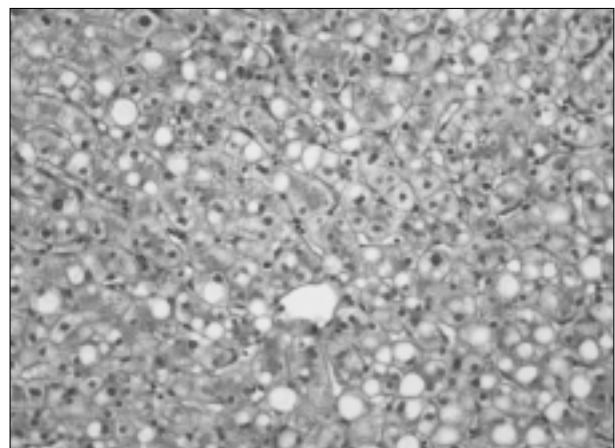
Screening for acute viral hepatitis was negative; indications of hepatitis A, cytomegalovirus, Epstein-Barr virus, or human immunodeficiency virus (HIV) infections were not present. Hepatitis B surface antigen was negative; antibodies against hepatitis C were not present. There was no evidence of an infection with *Brucella abortus bovis*, *Treponema pallidum* or *Toxoplasma gondii*. Levels of copper and ceruloplasmin were normal. Antinuclear antibodies, antineutrophil cytoplasmic antibodies, anti-liver-kidney microsomal antibodies and antimitochondrial antibodies were within normal limits.

Since the cause of the liver enzyme disturbances was uncertain, a liver biopsy was performed. The biopsy



**Figure 1**  
X-Y plot of the evolution of AST and ALT in our patient with breast cancer

AST = aspartate aminotransferase, ALT = alanine aminotransferase. The Y-axis indicates the value of AST and ALT in units per litre (U/l). The X-axis indicates the date on which these parameters were determined. On 14/06, treatment with CMF was started. The arrow indicates the cessation of treatment with methotrexate and the replacement by mitoxantrone. This replacement is followed by a normalisation of the liver function tests.



**Figure 2**  
Liver biopsy demonstrating pericentral steatosis, moderate hepatocellular anisonucleosis, hepatocellular swelling (ballooning) and the presence of lipofuscinosis, pointing towards reversible, possibly toxic liver cell damage (Roenigk grade II) - final magnification x 200

specimen contained liver tissue with signs of pericentral liver steatosis and evanescence of hepatocytes in pericentral areas. The pathological findings were compatible with toxic liver damage (figure 2). There was no histological evidence of liver metastases.

Because a toxic hepatitis due to methotrexate was considered to be the aetiology of the liver enzyme disturbances, this drug was stopped and replaced by mitoxantrone (10 mg/m<sup>2</sup>). The liver tests and CA 15.3 returned to normal values afterwards. Seventeen months after diagnosis, there is no evidence of metastatic disease and the liver function tests and tumour marker remain within normal limits.

## DISCUSSION

Methotrexate (amethopterin) is the 4-amino, N<sup>10</sup>-methyl analogue of folic acid. It is an inhibitor of the enzyme dihydrofolate reductase (DHFR), responsible for the maintenance of the intracellular folate pool in its fully reduced form as tetrahydrofolates. These folates are single carbon unit carriers and play a role in the synthesis of thymidylate, a precursor of DNA, and the purines adenosine and guanosine, precursors of both DNA and RNA. Therefore, it acts as a chemotherapeutic agent by DNA (and RNA) synthesis inhibition.

The folic acid analogues were introduced in 1948 by Farber *et al.* as antineoplastic agents for the treatment of children with acute lymphoblastic leukaemia (ALL).<sup>1</sup> A variety of dose schedules have been explored since then.<sup>2</sup> Nowadays, methotrexate is used in the treatment of breast cancer, osteosarcoma, head and neck cancer, choriocarcinoma, urothelial cancer, lung cancer, ALL and non-Hodgkin's lymphoma (NHL). It is also used to treat a variety of non-malignant diseases, including psoriasis, rheumatoid arthritis (RA), juvenile rheumatoid arthritis, dermatomyositis, Wegener's granulomatosis, sarcoidosis or bacterial and parasitic infections associated with HIV. Furthermore, reports on the use of this agent in the treatment of inflammatory bowel disease have been published.<sup>3</sup> The drug has even been used in the treatment of patients with autoimmune hepatitis<sup>4</sup> or idiopathic granulomatous hepatitis.<sup>5</sup>

## ADVERSE REACTIONS

Toxicity of methotrexate mainly consists of myelosuppression and mucositis. The elimination of the drug primarily occurs through renal excretion and toxicity is therefore more pronounced in patients with reduced renal function.<sup>6</sup> In rats with decreased renal function treated with high-dose schedules, methotrexate may cause renal impairment by

tubular necrosis due to crystallisation of 7-OH-methotrexate in the kidney tubules.<sup>7</sup> The renal toxicity in humans treated with methotrexate is thought to result from the same intratubular precipitation of MTX and its metabolites.<sup>8</sup> Acute systemic hypersensitivity reactions during initial administration (pruritus, urticaria and angio-oedema) have been reported<sup>9</sup> as well as late anaphylaxis after high-dose methotrexate.<sup>10</sup>

Other adverse reactions include methotrexate-induced pneumonitis<sup>11</sup> and neurological side effects<sup>12</sup> while numerous teratogenic effects of the drug have been demonstrated.<sup>13</sup> Life-threatening and even fatal toxicities have been described, in particular with high-dose regimens.<sup>14</sup>

## HEPATOTOXICITY

Methotrexate is also associated with acute and chronic liver damage. Acute elevations of hepatic enzyme levels and/or hyperbilirubinaemia are seen in more than 50% of the patients treated with a high-dose regimen, but a normalisation of the liver function tests is expected within one or two weeks after treatment.<sup>14</sup> Chronic administration of methotrexate (e.g. patients with psoriasis) can be complicated by liver fibrosis. An evolution to cirrhosis is possible.<sup>15</sup> Intermittent administration is associated with a lower incidence of liver function disorders.<sup>16,17</sup> Severe hepatotoxicity rarely occurs.

## MECHANISM OF TOXICITY

After intravenous administration, the half-life of methotrexate in plasma amounts up to 4.2 hours.<sup>18</sup> Methotrexate is possibly transported in hepatocytes by an active, sodium-dependent process.<sup>19</sup> After saturation of the active transport mechanisms, intracellular diffusion of methotrexate depends on the concentration gradient.<sup>20</sup> Methotrexate is then metabolised to polyglutamyl derivatives.<sup>21</sup> These conjugates may persist in liver cells and lead to prolonged inhibition of dihydrofolate reductase, resulting in a reduction of the folate coenzyme pool.<sup>21,22</sup> Schalinske *et al.* demonstrated a 70% reduction of the folate coenzyme pool after methotrexate treatment in rats.<sup>23</sup> This reduction has been shown to alter carbon flow through the hepatic folate dependent one-carbon pool. However, it is unclear whether this might play an important role in methotrexate-related hepatotoxicity. More likely, the formation of 7-OH-methotrexate after metabolism of MTX plays a role in this species.<sup>7</sup> Translation to the human situation is, however, unclear as the elimination pathways of MTX and 7-OH-MTX in rats are different from those in humans, i.e. mainly biliary in rats and mainly renal in humans.

The effect of methotrexate therapy on Ito cells (fat-storing,

vitamin A-containing stellate cells) has been studied by Hopwood and Nyfors in psoriatics treated with methotrexate.<sup>24</sup> They found a statistically significant increase in the number of these cells after MTX therapy. Ito cells can be transformed into myofibroblasts, capable of secreting collagen. This collagenisation consequently leads to liver fibrosis.<sup>25</sup> Whether this mechanism plays a role in the occurrence of methotrexate-related hepatotoxicity in cancer patients treated with intermittent methotrexate regimens, is unknown. In conclusion, the exact mechanism of MTX-induced hepatotoxicity is as yet unclear.

#### DETECTION OF MTX HEPATOTOXICITY

Although standard biochemical tests of liver function (AST, ALT, bilirubin, AP, prothrombin time or albumin) are not regarded as a reliable screening method in general,<sup>26,27</sup> some studies in psoriatic patients treated with methotrexate did find significant correlations between biochemical tests and histological findings.<sup>28-30</sup> O'Connor and colleagues were able to predict abnormal liver biopsy findings after methotrexate therapy, based on the following variables: age, gender, AST, AP, history of cholecystitis and cumulative dose of methotrexate (in grams).<sup>30</sup> The probability of concordance between the predicted probability and the observed response was 0.92. The joint sensitivity of AST, AP and total bilirubin to detect abnormal results from a post-treatment liver biopsy specimen in psoriatic patients was 0.86. The predictive value of three negative tests (AST, AP and total bilirubin) was 0.93. Serial elevations in transaminases are thought to be a better predictor of liver damage.<sup>31-33</sup> In our patient, a progressive increase in AST, ALT, AP and  $\gamma$ -GT levels was observed, suggesting a progressive liver injury. The antipyrine clearance,<sup>29</sup> galactose elimination capacity,<sup>28,34</sup> aminopyrine breath test<sup>34</sup> and bromosulphophthalein excretion test<sup>28</sup> are unreliable methods of detecting early stages of MTX-induced liver disease in patients treated with low-dose regimens. These tests can, however, be significantly impaired in high-dose regimens.<sup>35</sup> The aminoterminal peptide of type III procollagen (P3NP), detected in serum by radioimmunoassay, may be an indication of liver fibrosis, as has been observed in psoriatic patients on prolonged MTX treatment.<sup>28</sup>

Some imaging techniques of the liver (radionuclide scan, ultrasound examination, computed tomography and magnetic resonance imaging) are not accurate in the prediction of early liver disease.<sup>36-38</sup> Therefore, these imaging techniques remain complementary in the diagnostic work-up of patients with elevated liver tests. The gold standard for the detection of MTX-induced liver

damage is performing a liver biopsy. The Roenigk histological score is generally used for the staging of these biopsies.<sup>26,32,39</sup> The Roenigk scoring system consists of the following grades.<sup>40</sup>

- Grade I** Normal; mild fatty infiltration, mild nuclear variability, mild portal inflammation
- Grade II** Moderate to severe fatty infiltration; moderate to severe nuclear variability; portal tract expansion, moderate to severe portal tract inflammation and piecemeal necrosis
- Grade IIIA** Mild fibrosis
- Grade IIIB** Moderate to severe fibrosis
- Grade IV** Cirrhosis

This scoring system is not sensitive enough to detect small changes in the degree of fibrosis. Therefore, new scoring systems have been developed which may be of particular interest for patients on prolonged low-dose methotrexate treatment for a non-malignant disease.<sup>33,41</sup> The semi-quantitative histological scoring system (SSS), developed by Chevallier and colleagues,<sup>41</sup> analyses the following four sites of fibrosis:

- Perisinusoidal space (PS, grade 0-2)
- Centrolobular vein (CLV, grade 0-2)
- Portal tract (PT, grade 0-3)
- Number and width of septa (number {NS}: graded 0-3; width {WS}: graded 1-5)

The total score is given by  $CLV+PS+PT+2x(WSxNS)$  and ranges from 0-35. SSS is normal from 0 to 1, reflects mild fibrosis between 2 and 4, moderate fibrosis between 5 and 10, and pre-cirrhosis ranges from 11 to 15. The diagnosis of cirrhosis is made when the score is higher than 15. Guidelines for monitoring liver toxicity in MTX-treated patients with psoriasis were published in 1972,<sup>42</sup> 1973,<sup>43</sup> 1982<sup>44</sup> and 1988.<sup>40</sup> Guidelines for monitoring hepatotoxicity in patients with RA were published in 1994.<sup>33</sup> It is evident that these guidelines are not applicable to patients with breast cancer because of the different dose regimens and many other confounding factors.

Guidelines concerning the surveillance of patients with breast cancer after primary therapy were reviewed by the American Society of Clinical Oncology in 1998.<sup>45</sup> However, as far as we know, no guidelines concerning the follow-up during treatment with chemotherapy have yet been published. Nevertheless, many chemotherapeutic agents alone or in combination may cause hypersensitivity reactions or direct hepatotoxicity, and altered liver function may alter drug metabolism and cause an increased risk of non-hepatic toxicity. Therefore, liver function tests should be performed regularly during treatment. If liver function disturbances persist, additional non-invasive imaging may reveal the underlying cause. A liver biopsy should in our

opinion be performed if imaging provides insufficient information and if the findings on biopsy have therapeutic implications. If a toxic hepatitis with apparent repercussion on the general performance status of the patient can be demonstrated, the treatment with methotrexate should be stopped. The liver biopsy in our patient only demonstrated a moderate fatty infiltration (Roenigk score II), but the repercussion on the general performance was more severe. After cessation of methotrexate, the asthenia in our patient disappeared; her performance status and liver function tests returned to baseline values. In contrast with psoriatics and patients with RA, where methotrexate is only stopped when a Roenigk IIIB or IV severity score is present on liver biopsy (Newman<sup>26</sup> and Kremer<sup>33</sup>), an earlier cessation of treatment with methotrexate (Roenigk II) may be necessary in patients with breast cancer. Higher-dose regimens are indeed used in these patients and a greater repercussion on their wellbeing can therefore be expected.

If risk factors for liver disease are present (abnormal baseline AST values, severe alcohol consumption, diabetes, hepatitis B and/or hepatitis C infection) before starting treatment with chemotherapeutic agents, the use of the least hepatotoxic agents should be considered.

Reactivation of hepatitis B during treatment with chemotherapy (methotrexate, etoposide, doxorubicin and other agents) has been reported.<sup>46</sup> Wong *et al.* described nine hepatitis B carriers with haematological malignancies (NHL, ALL) who developed an exacerbation of hepatitis during (one patient) or after completion (eight patients) of chemotherapy (among which methotrexate), which was fatal in six.<sup>47</sup>

#### RISK FACTORS FOR INCREASED METHOTREXATE TOXICITY

Methotrexate is bound to albumin for 50 to 60%.<sup>32</sup> Hypoalbuminaemia can therefore result in increased levels of free methotrexate and as a consequence, toxicity might increase.<sup>14,48</sup> Similarly, toxicity will increase in case of renal dysfunction as a result of a decreased methotrexate excretion. Low serum folic acid levels are known to increase the risk of methotrexate toxicity.<sup>48</sup> Methotrexate accumulates in third-space fluid collections, such as ascites and pleural effusions. Slow release of MTX from these third spaces also leads to increased toxicity.<sup>8,49</sup> Transfusions with red blood cells immediately after MTX administration could increase the risk for side effects from MTX.<sup>49</sup> In addition, apart from the dose of MTX used,<sup>26,50,51</sup> obesity,<sup>26</sup> amount of alcohol intake<sup>50</sup> and age<sup>48,52</sup> may also contribute to the development of liver damage.

#### INCIDENCE OF METHOTREXATE HEPATOTOXICITY IN PATIENTS WITH BREAST CANCER

In the study by Vaughan *et al.*, 17 out of 21 patients with breast cancer and normal baseline AST levels developed an AST elevation at some time in the course of their treatment with CMF. Cyclophosphamide 100 mg/m<sup>2</sup>/d was given orally on days 1 to 14, methotrexate 40 mg/m<sup>2</sup> i.v. on days 1 and 8, and 5-fluorouracil i.v. on days 1 and 8; the treatment cycle was repeated at 28-day intervals. The range of highest AST values was 22-49 IU/l (upper limit of normal = 19 IU/l). Ten of the 16 patients with a normal baseline value of AP before chemotherapy developed an elevation during chemotherapeutic treatment (62.5%). Four patients developed defects on liver scan accompanied by elevated AP and AST levels. Liver biopsies only revealed focal congestion, non-specific inflammation, fat deposition and no liver metastases. After cessation of chemotherapy, the hepatic lesions on liver scan improved or resolved completely. Nearly 50% (11/24) of the patients received 24 cycles of CMF.<sup>36</sup> The number of patients included was limited and the elevation of the liver function tests was only mild in most of these patients. Liver biopsies were not always performed.

Bajetta *et al.* studied the incidence of liver damage in 802 women with breast cancer. The patient population was randomised to receive either 6 or 12 cycles of adjuvant CMF (n=632). The dose of methotrexate was 40 mg/m<sup>2</sup> on days 1 and 8 of each monthly cycle. The control group consisted of women with breast cancer, only treated with a radical mastectomy (n=170). No increased incidence of abnormal liver tests with 6 or 12 cycles CMF was found compared with patients not receiving CMF (3.2% versus 4.1%). Liver biopsies (n=22) only revealed aspecific histological changes. Treatment with chemotherapy was never discontinued.<sup>53</sup> Statistical analysis was, however, not performed in this study, a historical control group was used. The definition of liver toxicity was insufficient and the time intervals at which liver function tests were performed during chemotherapy were not specified. Moreover, a liver biopsy was only performed in 22 patients, which makes the conclusions derived from these biopsies unreliable because of selection bias. Therefore, the incidence of liver function disturbances after MTX treatment is in all probability much higher than suggested by the latter article. These studies nevertheless suggest that, although elevated liver function tests can occur during methotrexate treatment in patients with breast cancer, the treatment regimen can be continued without increasing the risk for serious hepatic side effects. Further studies are needed to assess these issues.

## DRUG INTERACTIONS AND HEPATO-TOXICITY OF OTHER COMPONENTS OF THE CMF REGIMEN

Dexamethasone has been reported to increase hepatotoxicity of MTX in children with brain tumours. The reason for this finding is unclear. Steroids are known to cause an enzyme induction in hepatocytes. Hence, an altered metabolism of MTX could be responsible for the increased hepatotoxicity.<sup>54</sup>

Cyclophosphamide administration rarely results in hepatic injury. However, Honjo *et al.* reported a transient elevation of aminotransferase serum levels in 43% of the patients treated with cyclophosphamide.<sup>55</sup> Whether the transient elevation of transaminases was due to the administration of cyclophosphamide is, however, difficult to prove. All the patients received a combination of antineoplastic agents, several of which are known to be hepatotoxic. At that time, screening for hepatitis C was not possible. Concomitant use of other drugs was not mentioned. The possible confounding factor of alcohol intake was not evaluated.

Hepatic toxicity is only rarely associated with a treatment with 5-fluorouracil. Mild and reversible hepatotoxicity is, however, more frequently observed in treatment regimens with 5-FU plus levamisole, but this seldom results in symptoms.<sup>56</sup>

The observed liver toxicity in our patient is therefore more likely to be caused by methotrexate. Liver function tests indeed returned to normal values after cessation of this drug and its replacement by mitoxantrone. The general performance status returned to baseline values.

## MITOXANTRONE

Mitoxantrone is an anthracenedione that has shown therapeutic efficacy in NHL, acute non-lymphoblastic leukaemia and advanced breast cancer.<sup>57,58</sup> Mitoxantrone is mainly myelotoxic. It can safely be used in patients with breast cancer and moderate hepatic dysfunction.<sup>59</sup> Because repetitive blood samples showed a progressive increase in transaminases in our patient, and a liver biopsy revealed a toxic hepatitis, methotrexate was stopped and mitoxantrone was given instead. In our protocol methotrexate was considered to be the most hepatotoxic drug. A recovery of clinical symptoms and a normalisation of the liver tests were seen after cessation of MTX, which indeed proves that the hepatotoxicity was due to the treatment with this drug. We believe that in case of MTX-associated hepatotoxicity, a replacement of this agent by mitoxantrone can be a valuable alternative in the adjuvant treatment of patients with breast cancer.

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