LEUKOPENIA DUE TO PARVOVIRUS B19 IN A CROHN’S DISEASE PATIENT USING AZATHIOPRINE

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ABSTRACT

Thiopurines such as azathioprine (AZA) and mercaptopurine (MP) are frequently used for the treatment of inflammatory bowel diseases (IBD). Patients with low or absent thiopurine S-methyltransferase (TPMT) activity, resulting in high 6-thioguanine nucleotide (6-TGN) levels, have an increased risk of developing leukopenia. Alternatively, certain viral infections could induce leukopenia. We present the case of an adult Crohn’s disease patient with a parvovirus B19 infection and leukopenia during long-term AZA therapy. The uncomplicated long-term use of adequately-dosed AZA and stable non-toxic metabolite levels could not acknowledge TPMT deficiency as a primary cause of the leukopenia. Parvovirus B19 was assumed to induce the leukopenia by restraining myeloid proliferation. In addition, AZA probably potentiated susceptibility to this viral infection and may have inhibited adequate immunological defence. Leukopenia during thiopurine therapy not explained by TPMT deficiency could be induced by parvovirus B19 infection and compels temporal but not permanent cessation of thiopurine therapy.
INTRODUCTION

In the treatment of inflammatory bowel diseases (IBD), thiopurines are well-established immunosuppressive drugs that effectively maintain remission and reduce the need for corticosteroids\textsuperscript{1,2}. Unfortunately, the therapeutic window of thiopurines is small and adverse events are common. During thiopurine therapy myelotoxicity is a well-known adverse event that can be explained by deficient thiopurine S-methyltransferase (TPMT) activity with subsequent high 6-TGN levels\textsuperscript{3}. As part of myelotoxicity, leukopenia may increase susceptibility to opportunistic infections. Conversely, several viral infections can also predispose to myelodepression. It can be difficult to determine whether thiopurine induced myelodepression provoked the viral infection or vice-versa. We present the case of a Crohn's disease (CD) patient who developed leukopenia following an acute parvovirus B19 infection during the long-term use of azathioprine. Moreover, we discuss pathophysiological mechanisms that could be involved.

CASE

In 1988, CD was diagnosed in a 12-year-old girl. The disease was located in the descending and sigmoid colon. Some years later she underwent a partial resection of the distal colon for persisting inflammatory and stricturing CD. In 2000, at the age of 24, azathioprine (AZA) was introduced to treat chronic anal ulceration and local rectal stenosis at a dose of 2.5 mg/kg bodyweight. In May 2007, she gave birth to a healthy child by caesarean section. At the end of 2008, she presented with a combination of rapid progressive symptoms. She complained about a tendency to collapse in combination with cold shivers and general malaise. In addition, she felt to have fever, although she failed to record her body temperature. Furthermore, non-itching skin eruptions appeared on her chest and finally, her right elbow was painful. She had no known allergy and CD appeared in ongoing remission based on clinical and laboratory evaluation. Nevertheless, she lost 3 kg of weight due to reduced appetite in the last months. The treatment of CD still consisted of AZA in an unchanged stable dose of 125 mg/day in combination with topical corticosteroids. For eight years, AZA treatment was well tolerated without the report of drug-induced adverse events during regular routine visits at the Outpatient Clinic. Moreover, thiopurine metabolites were stable over time and below toxic levels, with mean values of 212 and 201 pmol/8x10\textsuperscript{8} RBC for 6-TGN and 6-MMP, respectively. Interestingly, shortly prior to the presentation of her symptoms, her recently born son experienced general malaise with skin eruptions that were similar to hers. The general practitioner diagnosed her son to have “fifth disease”, also known as erythema infectiosum, which is caused by a parvovirus B19 infection. Physical examination revealed a lean woman with a weight of 53 kg and BMI of 17 kg/m\textsuperscript{2}. Vital functions including body temperature were normal. Several skin eruptions consisting of erythematous, oedematous plaques were present on her chest. Further physical examination was normal; in particular no signs of arthritis were present. Laboratory tests revealed a leukopenia without other...
striking abnormalities (table 1). Additional serological tests revealed not only IgG antibodies, but also IgM antibodies against the parvovirus B19, reflecting a recent infection with the virus that supposedly caused erythema infectiosum in her child. While as the parvovirus B19 infection was assumed to have induced leukopenia, AZA therapy was interrupted to potentiate immunological defence. The parvovirus B19 infection with leukopenia and skin eruptions resolved after the cessation of AZA. Several weeks after her recovery, AZA was successfully reintroduced without - as anticipated - causing leukopenia.

**DISCUSSION**

**Thiopurines and myelodepression**

The occurrence of side effects is a major drawback in the use of thiopurines, such as AZA and mercaptopurine (MP). These side effects can be roughly subdivided in dose-dependent side effects and dose-independent or idiosyncratic side effects. Of the former, myelotoxicity is a well-known adverse event that has an incidence rate of 3% (95% CI 3-4%) and can be caused by elevated concentrations of the pharmacologically active 6-TGNs. In this respect, the median 6-TGN level in a leukopenic subgroup of IBD patients was higher compared to IBD patients without leukopenia. Thiopurine S-methyltransferase (TPMT) deficiency, which brings about high 6-TGN levels, is an important underlying cause of leukopenia during thiopurine therapy. At present, more than 20 variant alleles have been identified that reduce TPMT activity. However, variation in TPMT activity is not the only risk factor for the development of myelotoxicity. Of 41 CD patients with leukopenia during thiopurine therapy, in only 11 (27%) variant TPMT alleles could be identified. Aside from high levels of 6-TGN, 6-methyl-mercaptopurine ribonucleoside (6-MMPR) levels above 10.000 pmol/8x10^8 RBC have also been correlated with myelotoxicity. These very high levels of 6-MMPR are thought to be due to ultra-methylation activity of TPMT. Recently, trinucleotide repeat variants in the promoter of the TPMT gene were identified, that correlated with this ultra-methylation activity. Although in this patient TPMT sequencing

<table>
<thead>
<tr>
<th>Parameter (reference value)</th>
<th>determined value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leukocyte count (4.10 x 10^9/L)</td>
<td>1.80</td>
</tr>
<tr>
<td>neutrophils (1.5-6.8 x 10^9/L)</td>
<td>1.10</td>
</tr>
<tr>
<td>lymphocytes (1.0-3.5 x 10^9/L)</td>
<td>0.31</td>
</tr>
<tr>
<td>monocytes (0.1-1.0 x 10^9/L)</td>
<td>0.29</td>
</tr>
<tr>
<td>basophils (0.0-0.2 x 10^9/L)</td>
<td>0.00</td>
</tr>
<tr>
<td>eosinophils (0.0-0.5 x 10^9/L)</td>
<td>0.11</td>
</tr>
<tr>
<td>Haemoglobin (8.5-11 mmol/L)</td>
<td>9</td>
</tr>
<tr>
<td>Thrombocytes (150-400 x 10^9/L)</td>
<td>155</td>
</tr>
<tr>
<td>C-reactive protein (0-8 mg/L)</td>
<td>9</td>
</tr>
</tbody>
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was not performed, the leukopenia was neither the result of decreased TPMT activity nor the result of increased TPMT activity as her 6-TGN and 6-MMP levels during standard dosed AZA (2.4mg/kg/day) were only 212 and 201 pmol/8x10^8 RBC, respectively. An additional cause of myelotoxicity, again by increasing 6-TGN levels, could be concomitant administration of certain drugs, such as 5-aminosalicylates and allopurinol that interfere with thiopurine metabolism. However, in our patient this was not the case.

Thiopurines and infection

It is generally known that myelosuppression increases the risk of infections. The cumulative incidence of infections among patients with thiopurine induced myelotoxicity is approximately 6.5%\(^4\). It has been suggested that neutrophils are preferentially being suppressed when leukopenia is induced by thiopurines, however, this finding is not a specific finding\(^10\). After organ transplantation, which constrains immunosuppressive therapy, cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are the most important viral pathogens causing a clinical spectrum that ranges from asymptomatic shedding to potentially life-threatening disease\(^11\). Conversely, viral infections can give rise to myelosuppression\(^3\). Recently, De Boer and colleagues described a case of pancytopenia induced by a CMV pneumonia in a CD patient with long-term use of AZA\(^12\). This case shows many similarities with that case except for the acute parvovirus B19 infection being the culprit in our case, causing a marked decrease of the white blood cell count, but leaving the red blood cell count unaffected.

Parvovirus B19 and myelotoxicity

Parvovirus B19 was unexpectedly discovered in the mid-1970’s by an Australian virologist while performing an assay for hepatitis B\(^13\). This virus is a small single-strand DNA-virus that lacks an envelope and belongs to a subgroup of erythroviruses that in turn belong to the genus Parvovirinae. Erythroviruses have extremely limited cell tropism and replicate predominantly in erythroid progenitor cells of burst-forming and colony-forming units\(^14\). The cellular receptor to which the viruses bind is the blood group P-antigen or globoside. The viral genome of the parvovirus B19 encodes only three proteins of known function. The two structural proteins, viral protein 1 (VP1) and viral protein 2 (VP2), are part of the viral capsid allowing the host’s immune system to identify them as pathogens. Parvovirus B19 is a common, global, self-limiting viral infection that is primarily transmitted by respiratory droplets. It is a common infection of childhood, as a result of which 50% of the 15-year-old children have specific anti-parvovirus B19 IgG antibodies\(^15\). Approximately 90% of all people over the age of 50 have these antibodies. The incubation time is around 10 days, with a peaking viral load at day eight or nine. The clinical presentation varies, in particular between adults and children. Generally, there are two distinct phases of infection. First, a non-specific phase starts at day seven or eight with complaints of headache, fever, myalgia and chills. Second, at day 17 or 18 a more specific phase commences with reticulocytopenia, a decreased haemoglobin concentration and a transient decrease in neutrophil, lymphocyte and platelet count. Although most cases of parvovirus B19 infection
are asymptomatic, the most common clinical presentation is erythema infectiosum, or "fifth
disease", that starts with flu-like symptoms followed by a characteristic exanthema on the face
named "slapped cheek" sign. Similar clinical findings were present in our patient's young son
several days before his mother presented with symptoms. In adults, arthropathy is one of the
most important complaints. Both the skin eruption and the arthropathy appear to be immune-
complex mediated and are usually absent if immunodeficiency is present.

The haematological manifestations of parvovirus B19 infections are of special interest.
The virus predominantly affects erythroid progenitor cells and viremia often gives rise to
inhibition of the red blood cell production, hence reticulocytopenia is common. Owing
to the long half-life of normal erythrocytes, this usually does not result in anaemia, as
was the case in our patient. However, in those patients with an increased erythrocyte
turnover due to haemolysis or blood loss, the interruption of erythrocyte production can
precipitate a transient aplastic crisis with severe anaemia. In addition, pancytopenia due
to haemophagocytosis has been described during parvovirus B19 infection in patients
with hereditary spherocytosis\textsuperscript{16}. Haemophagocytosis is characterized by macrophages
phagocytosing different cell types in the bone marrow. Parvovirus B19 is generally self-
limiting. However, chronic infections have been reported in immunosuppressive states
which lack adequate anti-parvovirus B19 antibody formation\textsuperscript{17,18}. Consequently, pure red
cell aplasia, a persisting anaemia associated with reticulocytopenia, may arise.

Interestingly, our patient presented with a leukopenia in which the neutrophil and
lymphocyte count were most prominently decreased. As the parvovirus B19 was originally
thought to only affect erythroid progenitor cells, this finding was unexpected. However, few
case reports have been published describing the occurrence of neutropenia in previously
healthy subjects with a parvovirus B19 infection, suggesting that inhibition of the myeloid
progenitor cells can occur\textsuperscript{19,20}. In fact, this is not surprising as the globoside receptors
to which parvovirus B19 binds, are also present on cardiac myocytes, white blood cells,
platelets and trophoblast\textsuperscript{14}. In addition, auto-immunity triggered by the parvovirus B19
infection has been proposed as an explanation for neutropenia\textsuperscript{21}. Moreover, underlying
haematological or immunosuppressive conditions have been reported in 15 of 26 patients
with parvovirus B19-associated leukopenia\textsuperscript{22}. Correspondingly, in this patient the use of
AZA is assumed to have had an important role. Furthermore, leukopenia in this patient
could also be attributed to an increased expenditure of white cells due to the acute infection.

\section*{CONCLUSION}
Thiopurines are known for their ability to induce myelotoxicity, which only in part can be
explained by a low TPMT activity with resulting high 6-TGN levels. Thiopurines interfere
with immunological functions and might make patients more susceptible to infections.
Besides high 6-TGN levels, viral infections themselves, including the parvovirus B19, can
induce or deteriorate a leukopenic event. We assume that thiopurine therapy is regular
unjustly and permanently withdrawn, whereas the actual cause is a limiting viral infection.
ACKNOWLEDGEMENT

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REFERENCES


