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Atypical haemolytic-uraemic syndrome in patient with metastatic colorectal cancer treated with fluorouracil and oxaliplatin: a case report and a review of literature

Giuseppe Viscardi,[1] Nicoletta Zanaletti,[1] Maria Giovanna Ferrara,[2] Antonello Sica,[2] Umberto Falcone,[2] Salvatore Guastafierro,[2] Umberto Bracale,[3] Dario Ribero,[4] Morena Fasano,[1] Stefania Napolitano,[1] Pasquale Vitale,[1] Vincenzo De Falco,[1] Emilio Francesco Giunta,[1] Erika Martinelli,[1] Davide Ciardiello,[1] Fortunato Ciardiello,[1] Teresa Troiani[1]

[1]Medical Oncology, Department of Precision Medicine, Università degli Studi della Campania Luigi Vanvitelli, Naples, Italy
[2]Oncohematology Unit, AOU Luigi Vanvitelli, Naples, Italy
[3]Department of Endocrinology, Gastroenterology and Endoscopic Surgery, Università degli Studi di Napoli Federico II, Naples, Italy
[4]Hepatobilio-Pancreatic and Colorectal Surgery Unit, Candiolo Cancer Institute-FPO IRCCS, Candiolo, Italy

Correspondence to
Dr Teresa Troiani;
troiani.teresa@yahoo.it

ABSTRACT

Background. Thrombotic microangiopathies (TMA) are relatively rare but severe disorders characterised by non-immune haemolytic anaemia, thrombocytopaenia and organ failure. In patients with metastatic cancer, sporadic forms of TMA can be triggered by chemotherapeutic agents or can occur as complication of malignancy itself or of infections.

Case report. Hereby, we report a case of a patient diagnosed with metastatic colorectal cancer who experienced an atypical haemolytic-uraemic syndrome (aHUS) during chemotherapy treatment with FOLFOX6 scheme. The use of eculizumab led to prompt recovery of laboratory parameters that was maintained despite treatment discontinuation due to appearance of pneumonia infectious. Additionally, genetic analyses revealed the presence in heterozygosis of CFH gene polymorphisms associated with aHUS.

Conclusion. This case emphasises the importance of considering TMA as a possible diagnosis in patients with cancer presenting with haemolytic non-immune mediate anaemia and thrombocytopaenia associated with worsening of renal function. Prompt diagnosis is crucial for the requirement of its specific treatment that can impact on long-term outcome and prognosis.

Key questions

What is already known about this subject?
► The most common forms of thrombotic microangiopathies (TMA) are thrombotic thrombocytopaenic purpura (TTP) and haemolytic-uraemic syndrome (HUS).
► They are rare but severe disorders characterised by endothelial cell activation and thrombus formation leading to non-immune haemolytic anaemia, thrombocytopaenia and organ failure.
► Triggers for atypical HUS (aHUS) sporadic form in oncological patients include infections and sepsis, disseminated malignancies or some anticancer drugs.

What does this study add?
► We report a case of aHUS developed in patient with metastatic colorectal cancer during FOLFOX treatment.
► We hypothesise that oxaliplatin could be responsible of this disorder through an immune-mediated mechanism.
► In addition, presence in heterozygosis of CFH gene polymorphisms associated with aHUS was identified in the patient.

BACKGROUND

Thrombotic microangiopathies (TMA) are relatively rare but severe disorders characterised by endothelial cell activation and thrombus formation leading to non-immune haemolytic anaemia, thrombocytopaenia and organ failure.[1] The most common forms of TMA are thrombotic thrombocytopaenic purpura (TTP) and haemolytic-uraemic syndrome (HUS).

Historically, the name atypical HUS (aHUS) has been referred to any HUS not due to Shiga toxin-producing *Escherichia coli* (STEC).[2] Triggers for aHUS sporadic form include infections and sepsis, disseminated malignancies or certain anticancer drugs, organ transplantation, pregnancy, immunosuppressant and antiplatelet agents. Approximately 50% of the sporadic cases, however, appear to be idiopathic.

Less than 20% of cases of aHUS occur in patients who have a family history of HUS. Genetic abnormalities have been documented in the familial form and also in the

sporadic (mainly idiopathic) form. They involve inactivating mutations of proteins inhibiting alternative complement pathway (CFH, MCP, CFI or thrombomodulin) or a gain-of-function mutation of the pathway activating factors (C3 or B factor) as well as anti-H factor IgG antibodies formation (CFH-Ab).

In TTP instead the underlying abnormality includes accumulation of ultralarge von Willebrand factor multimers resulting from a deficiency of a cleaving protease found in the plasma of normal individuals termed ADAMTS13.[3] In 70%–80% of patients, ADAMTS13 deficiency is acquired and is caused by the presence of circulating autoantibodies. In rare cases (about 10%), mutations in the *ADAMTS13* gene cause congenital deficiency of the protease and result in familial recessive form of TTP.

In patients with cancer, it is difficult to identify possible causes of TTP/HUS onset.[4] The role of chemotherapeutic agents in triggering TMAs have been reported following treatment with either single agents or combination regimens including mitomycin C, gemcitabine, cisplatin, oxaliplatin, docetaxel, bevacizumab, bortezomib.[5]

We hereby present a case of HUS developed in patients with metastatic colorectal cancer following FOLFOX6 (oxaliplatin, leucovorin and 5-fluorouracil (5-FU)) treatment.

CASE REPORT

In September 2016, a woman aged 55 years presented with rectal bleeding. Her medical history was relevant for blood hypertension and hypercolesterolaemia. List of medications included a calcium channel blocker and simvastatin.

Pancolonoscopy showed a circumferential sigmoid lesion. The biopsy revealed an invasive well-differentiated adenocarcinoma. Total-body CT scan showed two meta-static liver lesions (segments VI and VII with a maximum size respectively of 36 and 23 mm). Subsequently, the histological exam from left hemicolectomy performed in October 2016 confirmed the diagnosis. According to the American Joint Commission on Cancer seventh edition, TNM staging was T3 N1a (positive lymph node ratio: 1/25) M1a. *KRAS* exon 2 mutation was detected.

After surgery in November 2016, the patient started first-line treatment with FOLFOX6 regimen (oxaliplatin 85 mg/m^2 intravenous, leucovorin 400 mg/m^2 intravenous over 2 hours and 5-FU bolus 400 mg/m^2 intravenous on first day, followed by 5-FU infusional 2400 mg/m^2 intravenous continuous infusion for 46 hours, every 2 weeks) plus bevacizumab. The angiogenesis inhibitor was interrupted after first cycle, due to appearance of right axillary and subclavian thrombosis, requiring anticoagulant therapy, and patient continued treatment with FOLFOX6 scheme.

A CT scan, performed in March 2017 after seven cycles of chemotherapy, revealed a partial response according to Response Evaluation Criteria in Solid Tumours V.1.1 (Recist V.1.1). So in June 2017 the patient underwent hepatic metastasectomy. After surgery, starting from July 2017, she resumed perioperative treatment with FOLFOX6.

A week after third cycle of postoperative chemotherapy, the patient was admitted to our Unit with asthenia and anorexia. Clinical examination revealed mild arterial hypertension, mild peripheral oedema and absence of neurological symptoms. Blood test showed moderate normochromic anaemia (haemoglobin (Hb)=78 g/L), thrombocytopaenia (platelets (Plt)=30 000/mm^3) and acute kidney injury (creatinine=1.98 mg/dL) (table 1). This was initially attributed to chemotherapy toxicity and patient underwent transfusion of red cells and intravenous fluids administration.

But haemolytic anaemia was suspected based on finding of increased value of lactate dehydrogenase (LDH) (1549 U/L), bilirubin (3.2 mg/dL) mostly indirect (2.1 mg/dL). No abnormalities were reported in coagulation tests. Further laboratory investigations revealed increased reticulocytes (6.48%), negative Coombs test, reduced haptoglobin (16 mg/day), presence of

Table 1 Serial blood results following admission

	Baseline	After plasmapheresis	Response to eculizumab	Follow-up
Haemoglobin (g/L)	78	77	101	112
Platelets (x10^6/mm^3)	30	55	84	145
Creatinine (mg/dL)	1.98	0.60	0.60	0.63
Lactate dehydrogenase (U/L)	1549	508	379	204
Total bilirubin (mg/dL)	3.2	2.72	0.96	0.87

schistocytes in the peripheral blood smear and proteinuria in nephrotic range (11133.6 mg/24 hours). C3 levels were slightly reduced with normal C4 levels. Stools cultures were negatives. No metastasis or infectious foci were proved at total body CT scan.

Given the suspicion of TMA, by day 3, steroid therapy (methylprednisone 1 mg/kg intravenous) and daily plasmapheresis/plasma infusion were started. After 2 weeks, renal function normalised but haematological recovery was minimal (table 1). Severe anaemia was still present; compared with the baseline Plt count was increased (Plt=55 000/mm^3) and LDH reduced (491 U/L) but without normalisation. Moreover, further serum analysis revealed normal ADAMTS13 metalloproteinase level (>10%).

These findings were consistent with diagnosis of aHUS. Therefore, decision to start treatment with eculizumab was taken.

For this purpose, meningococcal vaccine, followed by antibiotic prophylaxis with ciprofloxacin for 2 weeks, was administered. Informed consent was signed. Eculizumab was administered at dosing of 900 mg intravenous weekly as induction for 4 weeks, followed by maintenance at 1200 mg intravenous every 2 weeks. Just after 2 weeks from the beginning of therapy there was a recovery of the haematological parameters (Hb= 101 g/L, Plt=84 000/mm^3) and normalisation of serum LDH (379 U/L), without requirement of transfusion support (table 1).

However, in March 2018 after five cycles of maintenance, treatment with eculizumab was interrupted due to the appearance of fever and cough (figure 1). Chest CT scan showed bilateral infectious pneumonitis that was treated with broad-spectrum antibiotic therapy for 10 days (piperacillin/tazobactam, linezolid and levofloxacin) achieving a complete resolution of symptoms.

In September 2018, at a follow-up of 30 weeks after eculizumab discontinuation, normal haematological parameters (Hb=137 g/L, Plt=163 000/mm^3) persist, without alterations of LDH and renal function (table 1). CT scan shows subcentimetric pulmonary nodules suspicious of metastases for which a close radiological monitoring was decided. Due to progressive dimensional increment of pulmonary lesions and appearance of hepatic metastases in February 2019, second-line treatment with

Figure 1 Chest CT scan showing pneumonia.

FOLFIRI (irinotecan 180 mg/m^2 intravenous, leucovorin 400 mg/m^2 intravenous over 2 hours and 5-FU bolus 400 mg/m^2 intravenous on first day, followed by 5-FU infusional 2400 mg/m^2 intravenous continuous infusion for 46 hours, every 2 weeks) was started. Until now, three cycles are been administered without appearance of haematological toxicities.

Finally, genetic analyses were carried out at 'Mario Negri' Institute laboratory. A next-generation sequencing through Ion Torrent platform of the entire coding region of the genes CFH, MCP, CFI, C3, CFB and THBD was performed on peripheral blood lymphocytes DNA. The presence in heterozygosis of some polymorphisms of CFH gene was highlighted, including c.-332C>T, c.2016A>G, c.2808G>T. The combination of these mutations identifies the CFH-H3 haplotype, which is associated with genetic forms HUS, and is present in heterozygosis in the patient.

DISCUSSION

Since 1973, reduced serum levels of complement fraction C3 with normal levels of C4 have been reported in patients with aHUS, reflecting complement activation and consumption, typically through alternative pathway.[6] Kidney is particularly susceptible to complement mediated damage; glomeruli and arterioles are typically affected, but other organs may be involved (brain, heart, lungs, gastrointestinal tract, pancreas). The histological lesions of HUS are characterised by thickening of arterioles and capillaries, endothelial swelling and subendothelial accumulation of proteins and cell debris. Increased Plt aggregation and thrombi affect the microcirculation. Haemolysis occurs, and schistocytes are evident in blood smears.[7] When non-treated, aHUS has a poor prognosis, with death rates as high as 25% and progression to end-stage renal disease in half the patients.

In our case, several clinical features help to distinguish HUS from TTP. Given the finding of non-immune haemolytic anaemia, thrombocytopaenia and kidney failure, the finding of normal levels of ADAMTS13 is consistent with diagnosis of HUS (figure 2). In addition, normal values of international normalized ratio (INR), partial thromboplastin time (PTT), fibrinogen, antithrombin III (ATIII) and D-dimer exclude a disseminated intravascular coagulation.

It has also been shown that baseline laboratory variables can be used as predictors of low ADAMTS13 activity. In a series of retrospective studies, patients with creatinine >1.7 mg/dL and Plt >30 000/mm^3 have a lower risk of having a deficiency of ADAMTS13 than those without these parameters.[8] In this case, negativity of stool culture for STEC suggests an atypical form. No evidence of metastatic disease was present at the time of presenting symptoms. So cytotoxic drugs may be more likely involved in the pathogenesis of the disease. Drugs may elicit a TMA through a direct toxic effect or an immune-mediated mechanism (by inducing the formation of antibodies
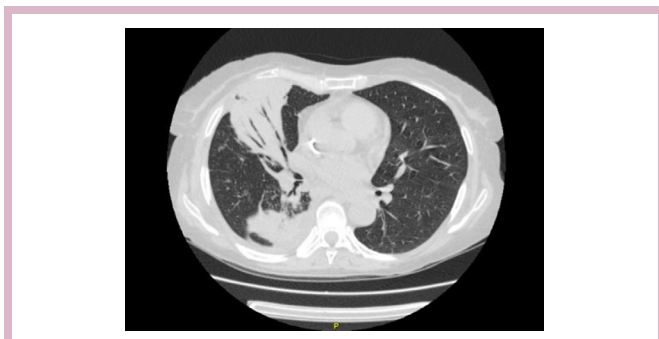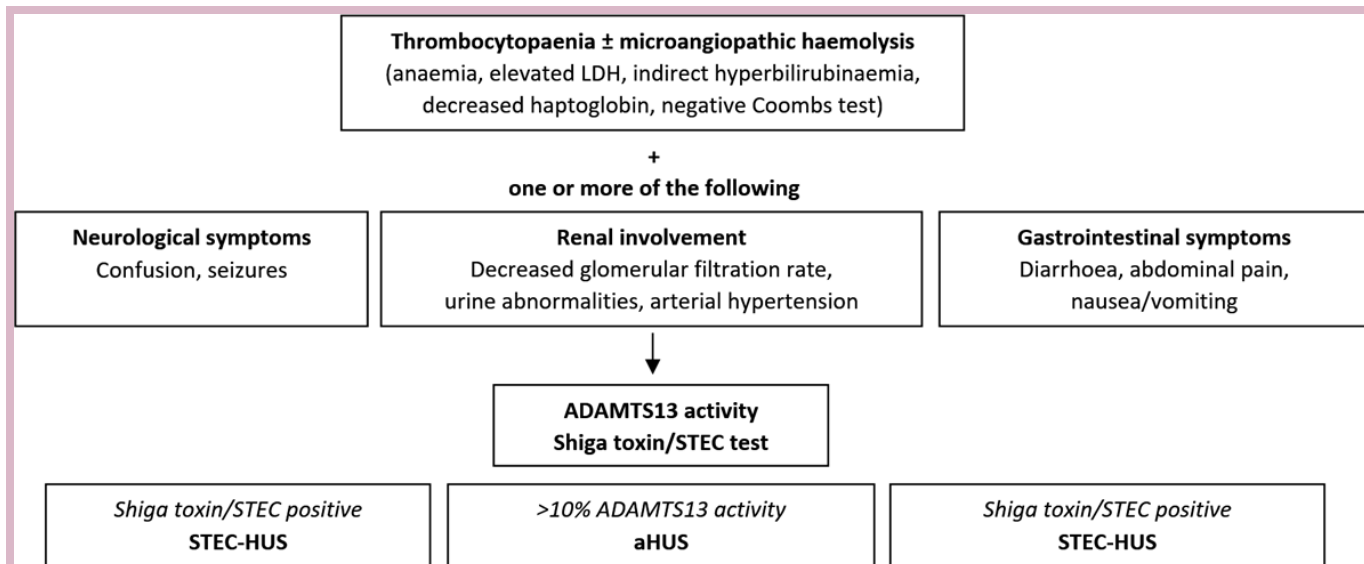
Figure 2 An algorithm for differential diagnosis of thrombotic microangiopathies. aHUS, atypical haemolytic-uraemic syndrome; LDH, lactate dehydrogenase; STEC, Shiga toxin-producing *Escherichia coli*.

against different cell types, including endothelial cells, causing immune complex formation, microvascular injury and Plt consumption).[9] Given the two-drug regimen (5-FU and oxaliplatin), it is difficult to attribute the disease to any single agent. However, several cases of TMA induced by platin salts are previously reported in literature.[10–13] We hypothesise that intermittent exposure to oxaliplatin in perioperative setting could be responsible for delayed onset of syndrome at moment of resumption, through the development of drug-dependent antibodies.

It is also known that common polymorphic variants of CFH gene may predispose to HUS.[14] In particular, CFH-H3 haplotypes include single nucleotide polymorphisms located in the promoter region of *CFH* that have potential functional implications in the expression of factor H, limiting the capacity to protect the cells from complement lysis.[15 16] In health, factor H is essential to maintain complement homeostasis. It accelerates the decay of the alternative pathway C3-convertase (C3bBb), binding to C3b or working as cofactor of factor I.[17] In our case, heterozygous haplotype CFH-H3 could have generated a situation of haploinsufficiency unable to provide sufficient protection to the cellular surfaces in a situation of complement activation. So polymorphic variants of gene could be involved in pathogenesis, likely through the action of triggers of oxaliplatin, which worked like a 'second hit'.

Prompt treatment for both TTP and HUS consists in plasmapheresis, and subsequently monoclonal antibody (rituximab in TTP and eculizumab in HUS) when diagnosis is defined.[18]

Complete response to plasmapheresis (recovery of Plt count, Hb and LDH accompanied by a reduction of creatinine by at least 25% compared with baseline) can be typically achieved in TTP, but not in aHUS.[19] It is reported that remission can occur after a median number of nine plasmapheresis.[20]

In our case, plasmapheresis did not produce a complete response. However, a certain degree of response can be observed in patients with HUS, also depending on the type of mutation possibly present, because the FFP contains sufficient amounts of CFH and/or plasmapheresis removes anti-CFH antibodies, producing a transient improvement.

Eculizumab, sold under the trade name of Soliris, is the first drug approved by the Food and Drug Administration and European Medicines Agency for the treatment of aHUS[21] and paroxysmal nocturnal haemoglobinuria.[22] It is a humanised monoclonal antibody that blocks the cleavage of terminal complement protein C5 into the inflammatory C5a protein and C5b, a precursor of the lytic C5b-9 complex. So eculizumab disrupts the terminal pathway of complement signalling reducing endothelial injury.

The majority of patients with aHUS treated with eculizumab achieve a complete response. In both of two prospective phase II trials, renal function improved (≥1 stage, 45%–65%) and haematologic parameters (LDH and Plt count) normalised (88%–90%), after 26 weeks of eculizumab therapy in patients with aHUS.[21]

However, optimal duration of treatment is not well defined. Discontinuing eculizumab treatment has been already described in small reports, with inconclusive results.[23] The rationale for discontinuation is to prevent serious side effects, such as meningococcal infections, and reduce costs. In our case, treatment was discontinued after 14 weeks due to appearance of pneumonitis. No subsequent haematological alterations were observed. However, the possibility of discontinuing eculizumab should be evaluated in large prospective studies.

In conclusion, even if HUS is a rare syndrome, it should be taken into account as a possible diagnosis in patients with cancer presenting with haemolytic non-immune-mediated anaemia and piastrinopenia, possibly associated with worsening of renal function. Prompt diagnosis is crucial for the requirement for its specific treatment, prognosis of disease acute phase and the long-term outcome.

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