CASE REPORT

Pathophysiology of tumour-induced microangiopathic haemolytic anaemia

Pavani Chalasani,¹ Jennifer M Segar,² Marilyn Marron,¹ Alison Stopeck³¹University of Arizona Cancer Center, Tucson, Arizona, USA²Department of Medicine, University of Arizona, Tucson, Arizona, USA³Stonybrook, Stony Brook, New York, USA**Correspondence to**Dr Pavani Chalasani,
pchalasani@uacc.arizona.edu

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SUMMARY

Cancer-associated microangiopathic haemolytic anaemia (CA-MAHA) is a syndrome characterised by Coombs-negative haemolytic anaemia and thrombocytopenia. It is primarily seen in advanced solid tumours and is distinct from thrombotic thrombocytopenic purpura/haemolytic uraemic syndrome. Diagnosis is often delayed and patients have a high mortality. We present the case of CA-MAHA in a patient with metastatic breast cancer treated successfully with early initiation of chemotherapy. In addition, we report longitudinal laboratory evaluation of circulating tumour cells and microparticles and suggest a hypothesis for the mechanism behind CA-MAHA.

BACKGROUND

Cancer-associated microangiopathic haemolytic anaemia (CA-MAHA) is a rare life-threatening complication characterised by non-immune haemolytic anaemia and thrombocytopenia. The prognosis of patients is poor despite treatment with anticancer therapies or plasmapheresis.¹ The pathophysiology that underlies CA-MAHA is not well understood, but is distinct from thrombotic thrombocytopenic purpura (TTP), haemolytic uraemic syndrome (HUS) and cancer-associated disseminated intravascular coagulation (CA-DIC). It is most frequently reported in patients with advanced stage cancers including breast, prostate, stomach and lung. CA-MAHA must be distinguished from other microangiopathic haemolytic anaemias including TTP, HUS and/or DIC, as therapies directed against these syndromes are ineffective in management of CA-MAHA.^{1 2} We describe a case of CA-MAHA in a patient with metastatic breast cancer (MBC), including successful treatment and correlative studies evaluating circulating tumour cells (CTCs) and microparticles (MPs), which may help in elucidating the pathophysiology behind CA-MAHA.

CASE PRESENTATION

A 69-year-old woman with initial diagnosis of stage II (T2N0M0) left breast invasive lobular cancer (ILC) in 1994, was treated with modified radical mastectomy and adjuvant chemotherapy (cyclophosphamide, methotrexate and 5-fluorouracil). She declined adjuvant hormonal therapy and did well until 2012, when she presented with increased urinary frequency. Work up including a cystoscopy revealed a bladder lesion that was biopsied and found to be a poorly differentiated metastatic carcinoma. On immunohistochemical analysis, the

tumour was cytokeratin 7 (CK7) positive, CK20 negative, estrogen receptor (ER) positive, progesterone receptor positive and human epidermal growth factor receptor 2 (HER2) was negative, consistent with MBC. She was also found to have a new right breast mass, which, on biopsy, revealed a high-grade ILC, ER+, negative HER2 and Ki 67 of 35%; epithelial cadherin (E-cadherin) was markedly diminished. Multiple enlarged right axillary lymph nodes and extensive bone metastases were noted on staging. The patient was started on combined therapy with anastrozole, fulvestrant and denosumab with stable disease as her best response.

After approximately 1 year on therapy, the patient developed abrupt onset of severe fatigue; laboratory evaluation revealed acute-onset anaemia (haemoglobin 10.5 g/dL, haematocrit 31%) and mild thrombocytopenia (75 000 cells/uL). She denied any fevers or cough and had no known contact with people who had been recently ill. During the course of the next 1–2 weeks, her anaemia and thrombocytopenia rapidly worsened leading to significant shortness of breath and worsening fatigue; she was hospitalised for further evaluation and treatment.

INVESTIGATIONS

Laboratory evaluation at time of admission revealed normocytic anaemia and severe thrombocytopenia (table 1). A peripheral blood smear revealed schistocytes and decreased platelets. Direct and indirect Coomb's tests were negative, cryoglobulins were undetectable, and liver and renal panels were unremarkable except for bilirubin level (table 1). Other laboratory work up for TTP and DIC were essentially negative, including a normal disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) level (table 1). Bone marrow biopsy showed dense bands of desmoplasia enveloping cytokeratin positive signet-ring cells, completely replacing the marrow space.

Correlative studies

CTCs were isolated from 2 mL of whole blood using the RosetteSep Human Circulating Epithelial Tumour Cell Enrichment Cocktail (StemCell Technologies). Enriched CTCs were collected in 2% fetal calf serum/1X Dulbecco's phosphate-buffered saline (D-PBS) and stained with (allophycocyanin, APC)-conjugated anti-CD45 (BD PharMingen), epithelial cell marker (PE)-conjugated antiepithelial cell adhesion molecule (EpCAM) (BD PharMingen), 7-aminoactinomycin (7AAD) (BD PharMingen) and fluorescein isothiocyanate (FITC)-conjugated

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Table 1 Comparison of laboratory values at time of presentation and after disease stabilisation

	2/4/14	1/8/15
WCC	6.3 K/uL	4.7 K/uL
Haemoglobin	7.6 g/dL	10.2 g/dL
HCT	22.1%	31.3%
Platelets	14 K/uL	150 K/uL
Reticulocyte count	7.21%	–
D-dimer	15.81 ug/mL	1.83 ug/mL
PT	13.8 sec	13.3 sec
INR	1.0	1.0
PTT	25.9 sec	25.9 sec
Fibrinogen	238 mg/dL	408 mg/dL
LDH	940 IU/L	271 IU/L
Haptoglobin	<8 mg/dL	57 mg/dL
CEA	1527.9 ng/mL	603.6 ng/mL
CA 27.29	2184.8 U/mL	958.2 U/mL
ADAMTS13	67%	
CTC estimate		
Cell search	7 CTC/7.5 mL	33 CTC/7.5 mL
EpCAM+		
>3 micrometres	12 290 cells/mL	289 cells/mL
>12 micrometres	6084 cells/mL	48 cells/mL
EpCAM+/Annexin V+/7AAD+		
>3 micrometres	49 cells/mL	44.6 cells/mL
>12 micrometres	41 cells/mL	8 cells/mL

7AAD, 7-aminoactinomycin D; ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; CA 27.29, cancer antigen 27.29; CEA, carcinoembryonic antigen; CTC, circulating tumour cells; HCT, haematocrit; INR, international normalised ratio; LDH, lactate dehydrogenase; PT, prothrombin time; PTT, partial thromboplastin time; WCC, white cell count.

Annexin V (Trevigen), in the presence of Annexin V Binding Buffer (Trevigen) prior to analysis by flow cytometry. CTCs were defined as the population of cells negative for leucocyte marker CD45, positive for EpCAM marker and gated by size to be greater than or equal to granulocytes to exclude smaller EpCAM positive cellular fragments or MPs. Early apoptotic CTCs were defined as Annexin V positive and 7AAD negative while late apoptotic/necrotic CTCs were double positive for both Annexin V and 7AAD.

MPs: Platelet-poor plasma (PPP) was prepared within 30 min after blood collection by centrifugation of whole blood at 1500g for 10 min at room temperature (RT). PPP was subsequently centrifuged at 13 000g for 10 min at RT generating platelet-free plasma (PFP). PFP samples were then diluted in 0.2 µM filtered 1X D-PBS (without calcium or magnesium) containing Annexin V Binding Buffer (Trevigen) and stained with (FITC)-conjugated Annexin V, peridinin-chlorophyll-protein complex (PerCP)-conjugated anti-CD45 and (APC)-conjugated CD41a. MPs were initially isolated by size using Megamix fluorescent beads (Biotex), and then cell of origin subtyped as platelet (PMPs)—Annexin V positive, CD45 leucocyte marker negative, CD41a platelet marker positive and CD62p positive if activated; endothelial cell (EMPs)—Annexin V positive, CD45 negative, CD41a negative and CD31 (platelet endothelial CAM, PECAM) positive; and monocyte (MMPs)—Annexin V positive, CD41a negative, CD45 positive and tissue factor (CD142) positive).

CTCs and MPs were analysed at time of presentation and after improvement, in an effort to understand the pathophysiology of CA-MAHA.

At presentation, we measured 6084 EpCAM-positive cells/mL in our laboratory. This level was the highest value ever observed in our laboratory in over 500 samples analysed from patients with cancer. Of note, the patient's blood was also sent for CTC quantification using the CellSearch (Veridex) analysis and returned at only 7 cells/7.5 mL.³ Once the patient's clinical presentation had stabilised, we repeated the studies—her circulating EpCAM positive cells had markedly decreased to 48 cells/mL, which correlated with clinical response and declining tumour markers. However, a repeat CTC estimate by CellSearch system reported an increase in her CTC count to 33 cells/7.5 mL. Interestingly, platelet, monocyte and EMPs estimates were significantly lower than normal controls in our laboratory, suggesting that circulating MPs might have been adherent to the high level of CTCs and tumour fragments.

DIFFERENTIAL DIAGNOSIS

Classic TTP was ruled out by the absence of clinical features of the classic pentad, including renal insufficiency, neurological symptoms and fever, as well as a normal ADAMTS13 level, and lack of response to plasmapheresis. Atypical HUS was also deemed likely secondary to normal renal function, and DIC was eliminated from the differential secondary to normal coagulation (prothrombin time and partial thromboplastin time) parameters.

TREATMENT

Tumour markers were significantly elevated (table 1) and, in the context of the patient's clinical and laboratory presentation, CA-MAHA was suspected, hence chemotherapy with weekly doxorubicin at 20 mg/m² and cyclophosphamide at 200 mg/m² (AC) was initiated. Plasmapheresis was also initially attempted but discontinued due to lack of improvement in clinical symptoms and laboratory abnormalities. The patient initially required red blood cell and platelet transfusions every 2 days but after three doses of weekly AC, her anaemia and thrombocytopenia improved and she eventually became transfusion-independent 12 weeks after initiation of chemotherapy.

OUTCOME AND FOLLOW-UP

The patient continues to do well 18 months after her presentation with this life-threatening scenario. After the initial few months on chemotherapy, her therapy was changed due to toxicity issues.

DISCUSSION

CA-MAHA is a rare complication of advanced stage tumours, and is associated with high morbidity and mortality. CA-MAHA in our patient was reversed after chemotherapy initiation and she continues to do well more than 1 year since her presentation, suggesting that early diagnosis and treatment initiation are critical to improve patient outcomes. Despite similar clinical presentations, treatments for TTP (fresh frozen plasma, plasmapheresis) are not effective for CA-MAHA.² Chemotherapy initiation with transfusion support during the acute presentation currently offers the only option for successfully treating the MAHA and prolonging survival.^{4 5}

One of the proposed hypotheses for CA-MAHA is triggering of the coagulation cascade by mucin released from the tumours.^{6 7} Immune mechanisms have been suggested in a few cases of CA-MAHA.⁸ Our correlative studies suggest that markedly elevated numbers of CTCs and, in particular, CTC fragments or cancer MPs, may be involved in the initiation of CA-MAHA. It is conceivable that tissue factor expressed on

tumour fragments and MPs resulted in activation of the clotting cascade and induced the observed microangiopathic picture. It is noteworthy that CTC quantification by the CellSearch system did not correlate with the number of CTCs measured by flow cytometry in our laboratory or with the patient's clinical course and outcome. However, longitudinal measurement of tumour

cell fragments did correlate with her outcome and indirectly supports our hypothesis that tumour cell fragments and MP may induce or propagate CA-MAHA in patients. As CellSearch only counts nucleated CTCs, CTC fragments are not accounted for and these fragments may play an important role in CA-MAHA as well as patient prognosis.

Patient's perspective

I felt as though I was very close to not surviving the anaemia. There were some very tense moments in the hospital. The treatment allowed me to leave the hospital and make it home, which was my ultimate goal. Since then, I had many months with a very good quality of life, for which I am very grateful.

Learning points

- ▶ Cancer-associated microangiopathic haemolytic anaemia (CA-MAHA) is a rare, life-threatening complication characterised by non-immune haemolytic anaemia and thrombocytopenia induced by the tumour.
- ▶ CA-MAHA must be distinguished from other microangiopathic haemolytic anaemias including thrombotic thrombocytopenic purpura, haemolytic uraemic syndrome and/or disseminated intravascular coagulation, as therapies directed against these syndromes are ineffective in management of CA-MAHA.
- ▶ Early recognition and treatment initiation during the acute presentation offers the only option for treating CA-MAHA.

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